IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte GERALD CAGLE, ROBERT L. ABSHIRE,
DAVID W. STROMAN, and JOHN M. YANNI

APPLICATION NO. 10/715,055
TECHNOLOGY CENTER 1600

METHOD OF TREATING OPHTHALMIC INFECTIONS
WITH MOXIFLOXACIN COMPOSITIONS

BRIEF ON APPEAL

Paul M. Rivard
Dale H. Hoscheit
Attorneys for Appellants
BANNER & WITCOFF, LTD.
1100 13th Street, N.W.
Washington, DC 20005-4051
(202) 824-3000 (telephone)
(202) 824-3001 (facsimile)

STATEMENT OF THE REAL PARTY IN INTEREST

The real party in interest is Alcon, Inc., the owner of the entire right, title, and interest in and to the subject application.

STATEMENT OF RELATED CASES

U.S. Patent 6,716,830,[1] the parent of the application under appeal, is involved in a litigation styled *Bayer Healthcare AG, Alcon, Inc. and Alcon Research, Ltd. v. Teva Pharmaceuticals USA, Inc.*, Civil Action No. 06-234 (SLR), pending in the U.S. District Court for the District of Delaware ("the Delaware litigation"). A trial was held in March 2008 but the district court has not yet issued a decision. This brief contains references to the trial transcript, which previously was made of record, in the format "Tr. [page: line] (witness)."

An opposition was filed in the European Patent Office (EPO) against EP 1,117,401 B, the European counterpart of the '830 patent. On October 30, 2006, the EPO issued a final decision favorable to the patentee, which has been appealed.

JURISDICTIONAL STATEMENT

The Board has jurisdiction over this appeal pursuant to 35 U.S.C. § 134. The claims under appeal were rejected in a Final Office Action mailed November 7, 2008. A Notice of Appeal was timely filed on February 6, 2009.

---

[1] The claims of the '830 patent are directed to ophthalmic formulations containing moxifloxacin, whereas the claims under appeal are directed to methods of treating ophthalmic infections by administering compositions containing moxifloxacin.

TABLE OF CONTENTS

TABLE OF AUTHORITIES

Page

No amendment after final rejection has been filed.

GROUNDS OF REJECTION TO BE REVIEWED

Claims 11-25, 27, 30, 32, 34, and 36-58 stand rejected under 35 U.S.C. §

103(a) as being obvious over Petersen et al. U.S. Patent 5,607,942 ("Petersen") in

view of Cagle et al. WO 90/01933 ("Cagle '933").

STATEMENT OF FACTS

1.   The subject matter under appeal is directed to a method of treating

ophthalmic infections by topically applying to the eye a composition containing

moxifloxacin or a pharmaceutically useful hydrate or salt thereof. Specification, p.

3, lines 3-5. Moxifloxacin is a fluoroquinolone and is described and claimed in

Petersen. Specification, p. 5, line 27 to p. 6, line 3.

2.   Petersen describes 151 quinolones[2] in Table I (columns 21-52) and 53

indications for which the disclosed compounds may be administered (column 54,

lines 7-22).   Petersen also refers to approximately 34 additional indications for

animal treatments (column 54, lines 23-46).   This amounts to more than eight

---

[2] Table I actually discloses well more than 151 compounds because enantiomers, diastereomers, and racemates are also described.   When enantiomers, diastereomers, and racemates are included, the total number of compounds depicted in Table I is approximately 400.

thousand (8,000) treatment combinations of quinolone structures and indications for human treatments, and more than thirteen thousand (13,000) treatment combinations when indications for animal treatments are included.

3.   In fact, Petersen does not correlate the dozens of disclosed treatment indications to the particular compounds listed in Table I, but rather to the entirety of the compounds encompassed by generic "Formula I." Thus, Petersen's description of treating eye infections actually encompasses an enormous number of compounds. See column 2, line 30 to column 3, line 25.

4.   A person skilled in the art would regard Petersen "to be general and potentially say that there may be a compound that could treat one or more of these infections." Tr. 968:2-975:4 (Zhanel).[3] The skilled artisan would not read Petersen as suggesting that each of the enormous number of compounds, or any particular one of them, can be used to treat each of the many, many listed indications. Tr. 295:8-296:8; 299:12-300:17; 285:22-291:9 (Allen)[4]; 974:25-975:4 (Zhanel).

---

[3] Dr. Zhanel testified in the Delaware litigation as an expert witness for the plaintiffs, Bayer and Alcon, on the issue of validity. Dr. Zhanel is a Professor in the Department of Medical Microbiology, Faculty of Medicine, University of Manitoba, Winnipeg, Canada. He advises government agencies in many countries regarding appropriate uses for antibiotics.

[4] Dr. Allen testified in the Delaware litigation as an expert witness for the defendant, Teva, on the issue of invalidity. He is Editor-in-Chief of the International Journal of Pharmaceutical Compounding, Chief Executive Officer of

5.   Moxifloxacin is not singled out in Petersen for any particular indication, and Petersen reports no *in vitro* or *in vivo* data of any kind for moxifloxacin. See Table I (column 58) and claim 2 (column 99). Petersen reports *in vitro* data for 18 fluoroquinolones as minimum inhibitory concentrations (MIC) (column 58). These data are of relatively little value to a person searching for improved ocular anti-infective therapies because (a) the spectrum of bacteria used in the testing is not the same as that typically used to evaluate anti-infective agents for ophthalmic use; notably absent is the most dangerous ocular pathogen *Pseudomonas aeruginosa*; and (b) there is no data for bacteria identified as quinolone-resistant, i.e., bacteria that have developed resistance to prior quinolones used in ophthalmology, particularly ciprofloxacin and ofloxacin, such as quinolone-resistant *Staphylococcus* species. Stroman Dec. ¶ 9.[5]

6.   Antibiotics are applied prophylactically during eye surgery, with a key focus on combating *Pseudomonas aeruginosa*, a dangerous ocular pathogen relative to infections that may cause a loss of sight. Stroman Dec. ¶¶ 4, 9(a). Ophthalmic infections are treated and prevented empirically, meaning that the identity of the pathogen causing the infection is not known when treatment or prophylaxis is

---

the Midwest Institute of Research and Technology, and Professor Emeritus at the University of Oklahoma HSC College of Pharmacy.

[5] Dr. Stroman is a co-inventor of the subject application and currently is Director of Anti-Infective Microbiology for Alcon Research, Ltd.

initiated. As a result, it is important that antibiotics in ophthalmic formulations have a broad spectrum of coverage against <u>all</u> important ocular pathogens, including and especially *Pseudomonas aeruginosa*. Alfonso Report at ¶¶ 33, 37[6]; Zhanel Report at ¶¶ 72.

7. Prior commercial anti-infective ophthalmic formulations employed certain other fluoroquinolones, with ciprofloxacin and ofloxacin considered to be the state of the art. See, e.g., Survey of Ophthalmology, International Review Journal, Vol. 50, Supp. 1, Nov. 2005 at S35-S36.

8. Bacteria are evolving species and are capable of developing resistance to anti-infective treatments. By the late 1990's, there was "acute" concern about resistance development in the area of ophthalmic infections "in light of escalating reports of ocular strains [including and especially *Pseudomonas aeruginosa*] that were resistant to currently-available quinolone therapy." Zhanel Report at ¶¶ 70-71; Alfonso Report at ¶ 37. Because ophthalmic anti-infective formulations stand as the last line of defense against serious ocular infections that may cause a loss of sight, this problem of quinolone resistance was considered "the most important

---

[6] Dr. Alfonso testified in the Delaware litigation as an expert for the plaintiffs on the issue of validity. He serves as Professor and the Edward WD Norton Chair in Cornea and External Diseases in the Department of Ophthalmology at the Bascom Palmer Eye Institute, University of Miami, where his work includes treating patients having complicated ophthalmic infections.

problem in ocular infectious diseases in September 1998." Tr. 877:2-24; 880:7-881:2 (Zhanel).

9. At the time of invention, resistance to antibiotics was understood to be "a class problem: a strain that develops resistance to one quinolone usually will be resistant or less susceptible to another quinolone." Zhanel Report at ¶ 71; Alfonso Report at ¶ 37. Because of these concerns, there was skepticism in the art about pursuing other quinolones for treating ophthalmic infections. Zhanel Report at ¶ 71 ("by 1998, a person of ordinary skill in the art would not have believed that another quinolone was the preferred solution to the growing problem of quinolone resistant ophthalmic infections."); Alfonso Report at ¶ 37 ("the solution to the problem was more likely to be found by looking to other (non-fluoroquinolone) classes of antibiotics.").

10. In light of the concerns surrounding quinolone resistance, to be considered a desirable candidate for anti-infective ophthalmic formulations, a quinolone would need to exhibit, at a minimum, improved activity relative to ciprofloxacin against gram-positive pathogens, such as *Staphylococcus aureus*, while maintaining or at least nearly maintaining ciprofloxacin's activity against important gram-negative pathogens, including *Pseudomonas aeruginosa*. Zhanel Report at ¶ 79.

11. However, at the time of invention, moxifloxacin was reported to be 2- to 8-fold <u>less</u> active than ciprofloxacin against *Pseudomonas aeruginosa* in *in vitro*

testing. Woodcock et al., Antimicrobial Agents and Chemotherapy, American Society of Microbiology, Jan. 1997. Because moxifloxacin not only failed to maintain ciprofloxacin's activity against *Pseudomonas aeruginosa* but exhibited *less* activity against *Pseudomonas aeruginosa* by a "hugely significant" amount, moxifloxacin was not regarded as a viable candidate for ophthalmic use at the time of invention. Tr. 860:14-861:1; 876:12-877:24 (Zhanel).

12.   Work by the present inventors yielded the surprising discovery that moxifloxacin, when administered topically to the eye as claimed in claim 11, is characterized by superior and unexpected ocular penetration properties that enhance its ability to treat ophthalmic infections. Stroman Dec. ¶ 13; Survey of Ophthalmology, International Review Journal, Vol. 50, Supp. 1, Nov. 2005 at S32 ("moxifloxacin penetrates ocular tissues better (two to three-fold) than gatifloxacin, ciprofloxacin, ofloxacin, or levofloxacin"); S36, Table 3 (moxifloxacin's ability to penetrate corneal tissue reported to be >2 to >9 times greater than that of the other fluoroquinolones tested); and S43, Table 10 (moxifloxacin's ability to penetrate conjunctivae tissue reported to be >6 to >14 times greater than that of the other fluoroquinolones tested).

13.   An embodiment of the present invention formulated as an ophthalmic solution containing 0.5 wt. % moxifloxacin (VIGAMOX®) has achieved very significant commercial success, reaching total global sales of $ 100,000,000 in its

first full year of sales (2004) and global sales of $ 185,000,000 in 2006. Stroman Dec. ¶ 21.

SUMMARY OF ARGUMENT

The § 103 rejection under appeal is an abstract, academic exercise entirely divorced from reality. The rejection relies on a very early, very general disclosure, itself lacking in sufficient particularity to form a rational basis for the rejection. This flawed reasoning is then compounded by ignoring intervening facts that definitively demonstrate the surprising and unexpected characteristics of the claimed invention, as well as facts demonstrating the skilled artisan would have been led away from the claimed invention.

Petersen claims the benefit of German applications filed in 1988 and a U.S. parent application filed in 1989 that issued in 1991 as U.S. Patent 4,990,517. The rejection applies the 1980's shotgun disclosure against the subject application having an effective filing date of September 30, 1998, almost ten years later, with no consideration whatever of real-world intervening events.

Ophthalmic formulations containing ciprofloxacin and ofloxacin were introduced in the 1990's and were commercially successful. Nonetheless, bacterial resistance to these agents was developing and became a cause for concern. Both ciprofloxacin and ofloxacin are, of course, fluoroquinolones as is moxifloxacin. The Final Office Action does not even attempt to explain why, given the concern

- 8 -

with bacterial resistance to the prior commercial fluoroquinolones – which was regarded as a problem shared by all quinolones – the skilled artisan should be led to consider yet another quinolone, much less one, such as moxifloxacin, that had been demonstrated to be *less* active than ciprofloxacin relative to a key ocular pathogen.

Nonetheless, despite these blazemarks that led in other directions, the present inventors made the surprising discovery that moxifloxacin would work exceptionally well for the treatment of ophthalmic infections. That discovery has enjoyed the endorsement of very significant commercial success – this as a result of the unpredicted and unexpected ocular penetration ability of moxifloxacin ophthalmic formulations, a property that is nowhere even hinted at in the prior art.

The Final Office Action, rather than addressing these compelling facts, simply chooses to ignore them. The selective use of an early shotgun disclosure is not a legitimate basis to reject the present claims in view of the intervening events that point the skilled artisan in an entirely different direction.

The weakness of the § 103 rejection is evident from the Examiner's own actions during the lengthy prosecution of this application. A first Office Action dated August 25, 2004 included §§ 102 and 103 rejections based on the same Petersen reference. After this first Office Action was withdrawn, <u>four</u> subsequent Office Actions were issued, none of which rejected any claims over Petersen. It

was not until April 4, 2007, nearly three years after the first Office Action, that Petersen was resurrected and again applied in a prior art rejection.

ARGUMENT

A. Petersen Does Not Describe Administering Moxifloxacin for Treating Eye Infections within the Meaning of the Patent Laws.

The Final Office Action asserts at page 2 that Petersen contains a "strong teaching" that moxifloxacin "is effective for the treatment of eye infection." Petersen contains no such teaching. Petersen discloses 151 different quinolone structures[7] in Table I (columns 21-52) and a laundry list of 53 indications for which antibiotics are generally administered (column 54, lines 7-22). Petersen also refers to approximately 34 additional indications for animal treatments (column 54, lines 23-46). This amounts to more than eight thousand (8,000) treatment combinations of quinolone structures and indications for human treatments, and more than thirteen thousand (13,000) treatment combinations when indications for animal treatments are included.

Moreover, Petersen does not correlate the dozens of disclosed treatment indications to the particular compounds listed in Table I, but rather to the entirety of the compounds encompassed by generic Formula I. See column 2, line 30 to column 3, line 25. Drs. Taylor and Zhanel testified for plaintiffs in the Delaware

---

[7] As explained in note 1 *supra*, Table I actually discloses well more than 151 compounds because enantiomers, diastereomers, and racemates are also described.

litigation that this generic formula discloses "billions" of compounds. Tr. 80:7-11 (Taylor); 968:23-25 (Zhanel). Moxifloxacin is not even among the "over a million" of "particularly preferred" compounds Petersen discloses at columns 3-4. Tr. 969:1-11 (Zhanel).

Although the rejection is made under § 103, it is premised on a reasoning of what Petersen "describes" that has long been rejected by the Federal Circuit and its predecessor courts. As Judge Rich explained in *In re Ruschig*, 379 F.2d 990, 994 (C.C.P.A. 1967), "something more than the disclosure of a class of 1000, or 100, *or even 48*" is needed for a generic disclosure to describe a particular combination (emphasis added). Rather, in order for there to be an adequate description, a prior art reference must "clearly and unequivocally disclose" the subject matter "without *any* need for picking, choosing, and combining various disclosures" in the reference. *In re Arkley*, 455 F.2d 586, 587 (C.C.P.A. 1972) (emphasis in original).

The Federal Circuit recently confirmed that the Final Office Action's approach of mixing and matching disclosures of a prior art reference is improper. In *Finisar Corp. v. DirecTV Group, Inc.*, 523 F.3d 1323, 1338 (Fed. Cir. 2008), the defendant relied on a chart that mapped the claim limitations to "various pages of" the reference. Noting the requirement that a prior art reference, to describe, "must disclose not only each limitation of the claim, but also each of those limitations

arranged as in the claim," the court held that the correlation of disparate disclosures from the reference to the claim elements was improper.

For at least these reasons, the Final Office Action is incorrect in its assertion that Peterson teaches that moxifloxacin is effective for the treatment of eye infections.

B.   Petersen Contains No Data Suggesting Ophthalmic Use For Moxifloxacin or Any of the Disclosed Quinolone Compounds.

Petersen provides no biological data for moxifloxacin and does not single out moxifloxacin for any particular indication, either in the claims or in the specification.   Petersen fails to disclose a single composition containing moxifloxacin, let alone a composition that could be used in the claimed topical ophthalmic treatment method.

Petersen's reference to treating eye infections with the quinolone compounds of Formula I, moreover, must be read in the context of the knowledge in the art at the time of invention – nearly ten years after the Petersen disclosure was written. Antibiotics are applied prophylactically during eye surgery, with a key focus on combating the dangerous ocular pathogen *Pseudomonas aeruginosa*.   Stroman Dec. ¶¶ 4, 9(a).   As ophthalmic infections are treated empirically without knowledge of the identity of the infective pathogen, it is important that antibiotics in ophthalmic formulations have a broad spectrum of coverage against all

important ocular pathogens, including and especially *Pseudomonas aeruginosa.* Alfonso Report at ¶ 72; Zhanel Report at ¶¶ 79-80.

At the time of invention, there were commercial fluoroquinolone ophthalmic formulations available, with ciprofloxacin and ofloxacin considered to be the state of the art. Persons skilled in the art searching for new quinolones sought, at a minimum, improved activity relative to ciprofloxacin against gram-positive pathogens, such as *Staphylococcus aureus*, while maintaining or at least nearly maintaining ciprofloxacin's activity against important gram-negative pathogens, including *Pseudomonas aeruginosa.* Zhanel Report at ¶ 79.

Petersen reports *in vitro* data for 18 fluoroquinolones as minimum inhibitory concentrations (MIC) (column 58). However, these data are of relatively little value for assessing the compounds' usefulness in treating ophthalmic indications. For one, the compounds were not assayed for inhibitory concentration against the dangerous ocular pathogen *P. aeruginosa.* Also, there is no data for bacteria identified as quinolone-resistant, i.e., bacteria that have developed resistance to prior quinolones used in ophthalmology, particularly ciprofloxacin and ofloxacin, such as quinolone-resistant *Staphylococcus* species. Stroman Dec. ¶ 9. Thus, there simply is nothing in Petersen that would have pointed persons skilled in the art toward selecting moxifloxacin for ophthalmic indications. *See In re Legator*, 352 F.2d 377, 378-80 (C.C.P.A. 1965) ("all properties of [the bactericide] must be

considered in light of all the requirements for a suitable agent when considering the question of obviousness of the claimed invention [i.e., the method of using the bactericide]").

C. The Prior Art as a Whole Taught Away From Selecting Moxifloxacin for Treating Eye Infections.

By the late 1990's, there was "acute" concern about resistance development in the area of ophthalmic infections "in light of escalating reports of ocular strains that were resistant to currently-available quinolone therapy." Zhanel Report at ¶¶ 70-71; Alfonso Report at ¶ 37.

Resistance to antibiotics was understood to be a class problem, leading to skepticism in the art that *any* quinolone would be effective in solving the growing problem of quinolone resistant ophthalmic infections. Zhanel Report at ¶ 71; Alfonso Report at ¶ 37.

At the very least, to be considered a desirable candidate at the time of invention, a quinolone would have needed to exhibit improved activity relative to ciprofloxacin against gram-positive pathogens, such as *Staphylococcus aureus*, while maintaining or at least nearly maintaining ciprofloxacin's activity against important gram-negative pathogens, including *Pseudomonas aeruginosa.* Zhanel Report at ¶ 79.

Yet moxifloxacin had been reported to be 2- to 8-fold less active than ciprofloxacin in terms of *in vitro* activity against *Pseudomonas aeruginosa.*

Woodcock et al., *Antimicrobial Agents and Chemotherapy*, American Society of Microbiology, Jan. 1997; Stroman Dec. ¶¶ 10-12. Although moxifloxacin had been reported as exhibiting good activity against *Staphylococcus aureus*, it was important for a candidate compound to be potent against <u>all</u> important virulent ocular pathogens (including and especially *Pseudomonas aeruginosa*), because treatment is administered without knowledge of the causative pathogen's identity. Dr. Zhanel refers to the prospect of using quinolones known to have relatively poor activity against an identified ocular pathogen as "discouraging." Zhanel Report at ¶ 72. In short, at the time of invention moxifloxacin was <u>not</u> regarded as a viable candidate for treating ophthalmic infections. Tr. 860:14-861:1; 876:12-877:24 (Zhanel).

Petersen also contains no data for bacteria identified as quinolone-resistant, i.e., bacteria that have developed resistance to prior quinolones used in ophthalmology, particularly ciprofloxacin and ofloxacin, such as quinolone-resistant *Staphylococcus* species. Stroman Dec. ¶ 9. Petersen certainly does not teach or suggest that moxifloxacin was a solution to the growing problem of quinolone resistance in ophthalmic treatments – and the rest of the literature available in 1998 clearly suggested it was not. As Dr. Zhanel testified at trial, "the art was teaching away from [using moxifloxacin to treat] pseudomonal infections.

It says anywhere you think *pseudomonas* is an issue, don't go there." Tr. 1017:6-1018:6, 1044:23-1045:17 (Zhanel).

A person of ordinary skill in the art would not have expected a composition containing moxifloxacin to solve the major concern in the field of ophthalmic infections – the rise of quinolone resistant *Pseudomonas*. Worse yet, skilled artisans would have expected use of moxifloxacin to exacerbate this problem and make the existing quinolone therapies less effective. It was well understood in the context of quinolones and *pseudomonas* that because "resistance occurs when you use an antimicrobial [and] you don't kill the pathogen, . . . using a drug that is weaker than other agents in the class," and thus less likely to kill the pathogen, "drive[s] resistance to not just this drug, but all of the drugs in that class." Tr. 886:1-887:23; 852:20-853:13 (Zhanel); 398:14-399:2 (Alfonso). A person of ordinary skill in the art, in view of moxifloxacin's significantly diminished *Pseudomonas* activity compared to ciprofloxacin, would have expected use of moxifloxacin in the eye "to drive quinolone resistance through the roof" and thereby "take the most important problem in ocular infectious diseases in September 1998, and by using a drug that won't kill *pseudomonas*, . . . make the problem worse." Tr. 876:12-877:24; 889:22-890:10; 897:6-21; 880:7-885:3 (Zhanel). It is for this additional reason that artisans in the field, with the exception of the inventors of the subject application, were not interested in

ophthalmic treatment using quinolones that, like moxifloxacin, were less active than ciprofloxacin against *pseudomonas*. Tr. 889:12-21 (Zhanel); 398:15-399:2 (Alfonso).

Thus, not only does Petersen fail to suggest the use of moxifloxacin for treating ophthalmic infections, but the prior art as a whole pointedly taught <u>away</u> from using moxifloxacin in anti-infective treatments – such as ophthalmic treatments – where *pseudomonas* is an important pathogen.

The Federal Circuit has held a claimed invention cannot be found obvious when persons skilled in the art would not have selected the compound on which the obviousness theory relies as a starting point. *Takeda Chem. Indus., Ltd. v. Alphapharm Pty. Ltd.*, 492 F.3d 1359, 1360-62 (Fed. Cir. 2007); *Ortho-McNeil Pharm., Inc. v. Mylan Labs., Inc.*, 520 F.3d 1358, 1364-65 (Fed. Cir. 2008). Because moxifloxacin was known to have relatively poor *in vitro* activity against the dangerous ocular pathogen *Pseudomonas aeruginosa* and because of concerns about creating *Pseudomonas* resistance, persons skilled in the art would <u>not</u> have been led to select moxifloxacin for treating ophthalmic infections. *See also Legator*, 352 F.2d at 378-80 (agreeing that using bactericide in claimed method was "a selection invention" that was non-obvious when all of the bactericide's properties were considered in view of needs in field and problem to be solved).

D. Administering Moxifloxacin Compositions for Treating Eye Infections Results in Superior and Unexpected Ocular Penetration Demonstrating the Non-obviousness of the Method of Claim 11.

Notwithstanding its relatively poor *in vitro* activity against *P. aeruginosa*, moxifloxacin in ophthalmic formulation was found to exhibit superior and unexpected ocular penetration properties enhance the ability to treat ophthalmic infections, including those caused by *Pseudomonas*. Stroman Dec. ¶ 13; Mitra Report at ¶¶ 57-65 (ocular pharmacokinetics of moxifloxacin formulation are characterized by "penetration rate into and concentration achieved in the ocular tissues [that] is very high and unexpectedly far superior to ofloxacin and ciprofloxacin . . .").[8]

The superior and unexpected ocular penetration properties of ophthalmic moxifloxacin formulations have been confirmed by third parties. A review of numerous published scientific articles, wherein the ocular bioavailabilities of moxifloxacin and other fluoroquinolones when administered via ophthalmic compositions are compared, is provided in Survey of Ophthalmology, International Review Journal, Vol. 50, Supp. 1, Nov. 2005. This article was authored by Alcon scientists, but the studies reviewed in the article were conducted by a variety of

---

[8] Dr. Mitra testified as an expert for the plaintiffs on the issue of validity. He is the Curators' Professor of Pharmacy and Chairman of the Division of Pharmaceutical Sciences at the University of Missouri in Kansas City. He is also Vice Provost for Interdisciplinary Research at the University of Missouri, and Director for Translational Research at the University of Missouri School of Medicine.

personnel, including both Alcon scientists and non-Alcon scientists. The overall significance of the results of the studies is summarized on page S32 of the Supplement as follows:

> The results consistently demonstrate higher maximum concentrations for moxifloxacin relative to the other fluoroquinolones in ocular tissues with levels well above its minimum inhibitory concentrations for relevant ocular pathogens . . . It is clear from the array of studies summarized in this report that moxifloxacin penetrates ocular tissues better (two to three-fold) than gatifloxacin, ciprofloxacin, ofloxacin, or levofloxacin. This consistent, enhanced penetration of topical moxifloxacin offers powerful advantages for ophthalmic therapy.

The same review article contains data showing moxifloxacin exhibits an even more pronounced (>2 to >9-fold) improvement over gatifloxacin, ciprofloxacin, ofloxacin, norfloxacin, levofloxacin, and lomefloxacin with respect to its ability to penetrate corneal tissue (Page S36, Table 3), as well as >6 to >14-fold improvement over gatifloxacin, ciprofloxacin, ofloxacin, and levofloxacin with respect to its ability to penetrate conjunctivae tissue (Page S43, Table 10).

The determination of whether an invention would have been obvious is a legal conclusion based on underlying factual inquiries including: (1) the scope and content of the prior art; (2) the level of ordinary skill in the prior art; (3) the differences between the claimed invention and the prior art; and (4) any objective

evidence of non-obviousness. *Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966).

In *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1734, 82 USPQ2d 1385, 1391 (2007), the Supreme Court explained, "While the sequence of these questions might be reordered in any particular case, the [*Graham*] factors continue to define the inquiry that controls." The Court said that "[t]he combination of familiar elements according to known methods is likely to be obvious when it does no more than yield <u>predictable</u> results." *Id.* (emphasis added).

The *KSR* Court relied heavily on predictability and specifically distinguished its earlier decision in *United States v. Adams*, 383 U.S. 39 (1966), where unexpected results led to a holding of non-obviousness. In *Adams*, the Court considered the obviousness of a wet battery that differed from the prior art in two respects. First, it contained water, rather than acids as conventionally used in storage batteries. Second, the electrodes were magnesium and cuprous chloride, rather than zinc and silver chloride. The invention was held to be non-obvious, despite the battery involving the substitution of elements that were known in the art.

> When Adams designed his battery, the prior art warned that risks were involved in using the types of electrodes he employed. The fact that the elements worked together in an unexpected and fruitful manner

supported the conclusion that Adams's design was not obvious to those skilled in the art.

KSR, 127 S. Ct. at 1740.

The facts of the present appeal are closely analogous to those in the *Adams* case. First, persons skilled in the art were skeptical that moxifloxacin would be effective for treating ophthalmic infections because of its relatively poor *in vitro* activity against the dangerous ocular pathogen *P. aeruginosa*. Stroman Dec. ¶ 12. Second, the present inventors made the surprising and unexpected discovery that compositions containing moxifloxacin are highly efficacious for treating ophthalmic infections, due to superior ocular penetration properties. As in *Adams*, the inventors' discovery of unexpected results in the face of skepticism compels a conclusion of non-obviousness.

The Final Office Action errs, *inter alia*, by failing to give proper consideration to the overwhelming record evidence of unexpected results. *In re Sullivan*, 498 F.3d 1345, 1358 (Fed. Cir. 2007) ("when an applicant puts forth relevant rebuttal evidence, as it did here, the Board must consider such evidence.").

E. The Final Office Action's Assertion Regarding a Lack of Criticality of the Claimed Concentration Range is Misplaced.

The Final Office Action asserts at page 2 that Appellants "allege[ ] criticality to the specific concentration range of the claimed composition, without showing

that the concentrations outside that range are not as effective." This argument is a *non sequitur* since Appellants are "not required to compare the claimed invention with subject matter that does not exist in the prior art." M.P.E.P. § 716.02(e). As discussed above, Petersen does <u>not</u> describe administering moxifloxacin (at any concentration) for treating ophthalmic infections, so a comparison between the claimed concentration range and "concentrations outside that range" is neither necessary nor appropriate.

The relevant comparison is between the claimed method and the closest prior art. *See, e.g., In re Chupp*, 816 F.2d 643 (Fed. Cir. 1987). Examiner Fay initially requested that claim 11 be amended to include the 0.1 to 1.0 wt.% concentration range to more fully distinguish Petersen, and later requested additional comparative data throughout the concentration range. The Owen declaration was prepared in response to this latter request. As Dr. Owen explains, "The superior results for moxifloxacin compositions are consistent across all of the tested drug concentrations tested (0.1, 0.3, 0.5, 0.75, and 1.0 wt.%)." Owen Dec. ¶¶ 9-12[9]; *See also* Mitra Report at ¶ 66 ("the superior penetration properties of moxifloxacin is present across the entire range of moxifloxacin concentrations (0.1% to 1.0%.) recited in claim 1 of the '830 patent."); Stroman Dec. at ¶ 17 ("these [superior ocular penetration] properties are prevalent over the entire range

[9] Dr. Owen has worked as an Alcon scientist since 1994 and currently holds the position of Technical Director, Pharmaceutical Research.

of 0.1 to 1%."). Thus, the present record amply demonstrates that the method of claim 11 is non-obvious over prior art methods involving ophthalmic administration of other fluoroquinolones.

F. Cagle Does Not Describe or Suggest Administering Compositions Containing Moxifloxacin, and Fails to Remedy the Deficiencies of Petersen.

The secondary reference, Cagle, is cited as describing ophthalmic formulations that contain steroidal and non-steroidal anti-inflammatory agents. The active agent is a fluoroquinolone such as ciprofloxacin, norfloxacin, ofloxacin, difloxacin, or pefloxacin (p. 1, lines 20-22). Cagle does not describe or suggest a method of treating ophthalmic infections by topically applying to the eye a composition containing moxifloxacin. Cagle thus fails to remedy the deficiencies of Petersen. Moreover, as discussed above, the present record demonstrates the non-obviousness of the claimed method over prior methods involving the administration of other quinolones, including ciprofloxacin and others described in Cagle.

CONCLUSION

Petersen does not describe administering moxifloxacin for treating ophthalmic infections within the meaning of the patent laws. Given the "shotgun" nature of the Petersen disclosure, its absence of biological data for moxifloxacin, its lack of data for any compounds suggesting ophthalmic use, and its total absence of any blazemarks leading to the choice of moxifloxacin for ophthalmic indications, Petersen fails on its own lack of adequate disclosure. What is more, the prior art taught away from selecting moxifloxacin for treating ophthalmic infections due to relatively poor *in vitro* activity against *P. aeruginosa*. Finally, the record contains overwhelming evidence of unexpected results that weighs heavily toward a conclusion of non-obviousness. Reversal of the obviousness rejection is respectfully requested.

Respectfully submitted,

ALCON, INC.

Date: April 6, 2009

By: /Paul M. Rivard/
Paul M. Rivard
Registration No. 43,446
Dale H. Hoscheit
Registration No. 19,090
Attorneys for Appellants
BANNER & WITCOFF, LTD.
1100 13th Street, N.W.
Washington, DC 20005-4051
(202) 824-3000 (telephone)
(202) 824-3001 (facsimile)

I. CLAIMS

1-10. (cancelled)

11. (rejected) A method of treating ophthalmic infections, which comprises topically applying to the eye a therapeutically effective amount of a pharmaceutical composition comprising moxifloxacin or a pharmaceutically useful hydrate or salt thereof in a concentration of 0.1 to 1.0 wt. % of moxifloxacin and a pharmaceutically acceptable vehicle therefor.

12. (rejected) A method according to claim 11, wherein the composition further comprises a steroidal or non-steroidal anti-inflammatory agent.

13. (rejected) A method according to claim 12, wherein the anti-inflammatory agent comprises a steroidal agent.

14. (rejected) A method according to claim 13, wherein the steroidal agent comprises a glucocorticoid.

15. (rejected) A method according to claim 14, wherein the glucocorticoid is selected from the group consisting of dexamethasone, rimexolone, prednisolone, fluorormetholone, hydrocortisone, mometasone, fluticasone, beclormethasone, flunisolide, triamcinolone, budesonide, and combinations thereof.

16. (rejected) A method according to claim 14, wherein the glucocorticoid comprises dexamethasone.

17. (rejected) A method according to claim 14, wherein the glucocorticoid comprises a 21-ether derivative of dexamethasone.

18. (rejected) A method according to claim 14, wherein the glucocorticoid comprises a 21-benzyl ether derivative of dexamethasone.

19. (rejected) A method according to claim 12, wherein the anti-inflammatory agent comprises a non-steroidal agent selected from the group consisting of prostaglandin H synthetase inhibitors, cyclooxygenase type II selective inhibitors, PAF antagonists, PDE IV inhibitors, and combinations thereof.

20. (rejected) A method according to claim 19, wherein the non-steroidal agent comprises a prostaglandin H synthetase inhibitor.

21. (rejected) A method according to claim 20, wherein the prostaglandin H synthetase inhibitor comprises nepafenac.

22. (rejected) A method according to claim 20, wherein the prostaglandin H synthetase comprises ketorolac.

23. (rejected) A method according to claim 20, wherein the prostaglandin H synthetase comprises diclofenac.

24. (rejected) A method according to claim 19, wherein the non-steroidal agent comprises a cyclooxygenase type II selective inhibitor.

25. (rejected) A method according to claim 11, wherein the composition is applied to the eye in connection with the treatment of a condition selected from the group

consisting of conjunctivitis, keratitis, blepharitis, dacyrocystitis, hordeolum, corneal ulceration, and combinations thereof.

26. (cancelled)

27. (rejected) A method according to any one of claims 11-24, wherein the composition is applied to the eye in connection with an ophthalmic surgical procedure.

28-29. (cancelled)

30. (rejected) A method according to any one of claims 11-25, wherein the composition contains moxifloxacin or a pharmaceutically useful hydrate or salt thereof at a concentration of about 0.35 wt. % of moxifloxacin.

31. (cancelled)

32. (rejected) A method according to any one of claims 11-25, wherein the composition is applied to the eye in connection with the treatment of conjunctivitis.

33. (cancelled)

34. (rejected) A method according to claim 30, wherein the composition is applied to the eye in connection with the treatment of conjunctivitis.

35. (cancelled)

36. (rejected) A method according to claim 11, wherein the composition is a liquid that contains sodium chloride.

37. (rejected) A method according to claim 36, wherein the composition further comprises at least one of a viscosity enhancing agent and a surfactant.

38. (rejected) A method according to claim 11, wherein the composition has a pH in the range of from 4.5 to 8.0.

39. (rejected) A method according to claim 38, wherein the composition has a pH in the range of from 5.5 to 8.0.

40. (rejected) A method according to claim 11, wherein the composition has an osmotic value compatible with the aqueous humor of the eye and ophthalmic tissue and in the range of from about 200 to about 400 milliosmoles per kilogram of water.

41. (rejected) A method according to claim 11, wherein the composition has an osmotic value compatible with the aqueous humor of the eye and ophthalmic tissue and in the range of from about 200 to about 300 milliosmoles per kilogram of water.

42. (rejected) A method according to claim 41, wherein the osmotic value is about 300 milliosmoles per kilogram of water.

43. (rejected) A method according to claim 11, wherein the composition contains a preservative at a concentration of from 0.001 to 1.0 wt. %.

44. (rejected) A method according to claim 11, wherein the composition is a sterile solution provided in a multi-dose form.

45. (rejected) A method according to claim 44, wherein the composition is applied to the eye in connection with the treatment of conjunctivitis.

46. (rejected) A method according to claim 32, wherein the composition inhibits the growth of *S. aureus* and contains moxifloxacin or a pharmaceutically useful hydrate or salt thereof in an amount sufficient to provide a moxifloxacin concentration in the lacrimal fluid and aqueous humor of the eye of at least about 0.13 micrograms per milliliter.

47. (rejected) A method according to claim 32, wherein the composition inhibits the growth of *S. epidermidis* and contains moxifloxacin or a pharmaceutically useful hydrate or salt thereof in an amount sufficient to provide a moxifloxacin concentration in the lacrimal fluid and aqueous humor of the eye of at least about 0.25 micrograms per milliliter.

48. (rejected) A method according to claim 45, wherein the composition inhibits the growth of *S. pneumoniae* and contains moxifloxacin or a pharmaceutically useful hydrate or salt thereof in an amount sufficient to provide a moxifloxacin concentration in the lacrimal fluid and aqueous humor of the eye of at least about 0.25 micrograms per milliliter.

49. (rejected) A method according to claim 11, wherein the composition is applied to the eye in connection with the treatment of keratitis and wherein the composition inhibits the growth of *P. aeruginosa* and contains moxifloxacin or a

pharmaceutically useful hydrate or salt thereof in an amount sufficient to provide a moxifloxacin concentration in the lacrimal fluid and aqueous humor of the eye of at least about 8 micrograms per milliliter.

50. (rejected) A method according to claim 32, wherein the composition inhibits the growth of *H. influenzae* and contains moxifloxacin or a pharmaceutically useful hydrate or salt thereof in an amount sufficient to provide a moxifloxacin concentration in the lacrimal fluid and aqueous humor of the eye of at least about 0.06 micrograms per milliliter.

51. (rejected) A method according to claim 11, wherein the composition is a sterile solution having a pH in the range of from 4.5 to 8.0; wherein the composition has an osmotic value compatible with the aqueous humor of the eye and ophthalmic tissues, including tissues that may have been compromised as the result of preexisting disease, trauma, surgery or other physical conditions, said osmotic value being in the range of from about 200 to about 400 milliosmoles per kilogram of water.

52. (rejected) A method according to claim 51, wherein the pH is from 5.5 to 8.

53. (rejected) A method according to claim 51, wherein the osmotic value is about 300.

54. (rejected) A method according to claim 51, wherein the solution also contains sodium chloride.

55. (rejected) A method according to claim 54, wherein the solution also contains at least one of a viscosity enhancing agent and a surfactant.

56. (rejected) A method according to claim 51, wherein the composition is applied to the eye in connection with the treatment of conjunctivitis.

57. (rejected) A method according to claim 55, wherein the composition inhibits the growth of at least one pathogen selected from the group consisting of *S. aureus*, *S. epidermidis*, *S. pneumoniae*, *P. aeruginosa*, and *H. influenzae*.

58. (rejected) A method according to claim 51, wherein the composition is applied to the eye in connection with an ophthalmic surgical procedure.

II.   CLAIM SUPPORT AND DRAWING ANALYSIS

This application contains no drawings and contains no claims in the means-plus-function format of 35 U.S.C. § 112, sixth paragraph.

An annotated version of independent claim 11, the only independent claim involved in this appeal, appears below.

11.   A method of treating ophthalmic infections **{page 3, lines 3-8}**, which comprises topically applying to the eye a therapeutically effective amount of a pharmaceutical composition comprising moxifloxacin or a pharmaceutically useful hydrate or salt thereof **{page 5, lines 25-27}** in a concentration of 0.1 to 1.0 wt. % of moxifloxacin **{page 7, line 15}** and a pharmaceutically acceptable vehicle therefor **{page 9, line 19}**.

III. EVIDENCE

IV. RELATED CASES

A final decision has not yet issued in the litigation *Bayer Healthcare AG, Alcon, Inc. and Alcon Research, Ltd. v. Teva Pharmaceuticals USA, Inc.*, Civil Action No. 06-234 (SLR).

A copy of the October 30, 2006 decision of the European Patent Office rejecting the opposition filed against EP 1,117,401 B is attached hereto.

⊠ EPA/EPO/OEB
D-80298 München

☎ +49 89 2399-0
TX 523 656 epmu d
FAX +49 89 2399-4465

**Europäisches
Patentamt**

**European
Patent Office**

**Office européen
des brevets**

Generaldirektion 2   Directorate General 2   Direction Générale 2

Best, Michael
Lederer & Keller
Patentanwälte
Prinzregentenstrasse 16
80538 München
ALLEMAGNE

LEDERER & KELLER
EINGANG / RECEIPT
3 1. 10. 2006
Erl: W.Vi! 30. Januar 2007.

Application No. / Patent No. 99 956 504.7 - 2123 / 1117401 /	Ref. 1732 EP F	Date 30.10.2006
Proprietor Alcon, Inc.		

Decision rejecting the opposition (Article 102(2) EPC)

The Opposition Division - at the oral proceedings dated 14.09.2006 - has decided:

The opposition(s) against the European patent EP-B- 1117401 is/are rejected.
The reasons for the decision are enclosed.

Possibility of appeal
This decision is open to appeal. Attention is drawn to the attached text of Articles 106 to 108 EPC.

Opposition Division:

Chairman:	ESTANOL, I
2nd Examiner:	Albrecht, Silke
1st Examiner:	TRIFILIEFF-RIOLO, S

LAUSENMEYER, J
Formalities Officer
Tel. No.: +49 89 2399-8074

Enclosure(s):   6 page(s) reasons for the decision (Form 2916)
                   Wording of Articles 106 - 108 (Form 2019)
                   Minutes of oral proceedings

to EPO postal service: 25.10.06

I   Summary of facts and submissions

1.   EP patent 1 117 401 is based upon EP patent application 99956504.7 filed on 29.09.1999 and claiming a priority date of 30.09.1998 from US 102504 and US 102506. The title is: Antibiotic compositions for treatment of the eye
The mention of the grant of the patent was published in EP Patent Bulletin 2003/47 of 19.11.2003.
Proprietor of the patent is Alcon Inc.

2.   Notice of opposition was filed by Teva Pharmaceuticals Industries on the 13.08.2004. The opponent requested revocation of the patent in its entirety because its content extends beyond the original application (A. 100(c)) and because its subject-matter lacks inventive step (A. 100(a)).
The opponent based his case on documents D1 to D5 (see Annex I).

A subsidiary request for Oral Proceedings was also made.

3.   In his reply to the notice of opposition, filed on the 28.06.2005, the proprietor requested maintenance of the patent as granted with correction of one obvious error on Page 3, presented arguments to this end supported by documents D6 to D11 (see Annex I) and made a subsidiary request for oral proceedings.

4.   Summons to oral proceedings were sent on the 23.01.2006. The opposition division expressed a provisional opinion according to which the requirements of A. 123(2) were met and inventive step would be discussed at the oral proceedings.

5.   In a letter dated 11.07.2006 the opponent sent further comments accompanied by two documents (D17 and D18, see Annex I).
He also added a ground based on the fact that the requirements of A. 54(5) were not met.

7.   On the 12.07.2006 the proprietor sent further comments and documents D12 to D16.

8.   On the 28.07.2006 the opponent objected that D12 to D16 should not be admitted

Entscheidungsgründe (Anlage)	Grounds for the decision (Annex)	Motifs de la décision (Annexe)

Datum
Date 30.10.2006
Date

Blatt
Sheet 2
Feuille

Anmelde-Nr.:
Application No.: 99 956 504.7
Demande n°:

because they were late filed and added that they were irrelevant.

9.   Some more comments were sent on the 29.08.2006 by the proprietor.

10.   Oral proceedings took place on the 14.09.06 at the end of which the opposition was rejected.

II Grounds for the decision:

1.   The requirements of A. 99, A. 100, R.1(1) and R. 55 are fulfilled. Therefore the opposition is admissible.

2.1. Objection under A. 123(2):

Claim 1 as granted concerns a topical ophthalmic composition comprising moxifloxacin or a hydrate or salt thereof in a concentration of 0.1 to 1.0 wt%.
In the application as originally filed the concentration of 0.1 to 1.0 wt% relates to "one or more compounds of formula I" (p. 7, l. 10-14 of the originally published version).
In this original application (published version) the general formula I is drawn on pages 4 and 13, moxifloxacin is drawn on page 5 and is not encompassed by the general formula I as drawn.
It is stated however in the description as filed that moxifloxacin is the "most preferred" compound (p. 5, l. 21) and that it is a compound of formula I (claim 6). Moreover, moxifloxacin is the only compound for which examples are provided, at a concentration of 0.3 and 0.35 wt%.
In view of this it is obvious that an error has occurred when drawing formula I in that a substituent (called A in the application as filed) has been omitted. Because of this omission, moxifloxacin is not encompassed by the general formula I whereas it is evident from the original application as a whole that it should have been, would substituent A have been correctly introduced in formula I.
Therefore when considering the original application as a whole, it is clear that compositions comprising moxifloxacin at a concentration of 0.1 to 1.0 wt% were meant to be part of the application.

| Entscheidungsgründe (Anlage) | Grounds for the decision (Annex) | Motifs de la décision (Annexe) |

Datum
Date 30.10.2006
Date

Blatt
Sheet 3
Feuille

Anmelde-Nr.:
Application No.: 99 956 504.7
Demande n°:

Thus the requirements of A. 123(2) are met.

2.2. Turning now to the correction of the formula on page 3 of the granted patent requested by the patentee: it consists in replacing the methyl group in position 3 of the quinoline ring by a carboxyl group. As stated in the description (p. 3, l. 1) this formula is intended to illustrate moxifloxacin. Since it can be assessed from any textbook that moxifloxacin has a carboxyl group and not a methyl group in this position, this correction is allowable under R. 88.

3.   Objection under A. 54(5).

According to the opponent the subject-matter of claims 1 to 11 contravenes the requirements of A. 54(5) because, as moxifloxacin is already known as an antibiotic (i.e. a medicament), the only means to claim its use as an ophthalmic agent is in the form of "further medical use" claims.

Article 54(5), however, deals with the so-called "first medical use" which allows to patent a substance for use in medicine, even if the substance per se is already known but provided that no "medical use" is known for it.

The subject-matter of present claims 1 to 11, however, is a specific topical ophthalmic composition comprising moxifloxacin in a specific concentration. It is not a claim aiming at protecting a medical use of moxifloxacin.

Thus no objection under A. 54(5) can be made for the subject-matter of claims 1 to 11 because the requirements of this article simply do not apply to this subject-matter.

4.   Objection under A. 56:

4.1. All parties agree to consider D3 as being the closest prior art document because it describes topical ophthalmic compositions comprising a quinolone antibiotic. The antibiotics cited are ciprofloxacin, norfloxacin, ofloxacin, difloxacin and pefloxacin (p. 1, l. 21, 22) with ciprofloxacin being singled out (see examples and claims).

Thus the difference with the subject-matter of the patent in suit is the antibiotic itself (moxifloxacin in the patent in suit).

The technical effect achieved by this difference lies in enhanced penetration properties in the eye tissues, as demonstrated by tests comparing moxifloxacin and ofloxacin submitted

| Entscheidungsgründe (Anlage) | Grounds for the decision (Annex) | Motifs de la décision (Annexe) |

Datum
Date 30.10.2006
Date

Blatt
Sheet 4
Feuille

Anmelde-Nr.:
Application No.: 99 956 504.7
Demande n°:

during the examination procedure (D11).

4.2. One of the opponent's arguments, however, is that the problem consisting in enhancing the penetration properties of an ophthalmic antibiotic is not stated in the original application which only mentions the need of enhancing the potency against ophthalmic pathogens as can be read on page 2, lines 4-6 (of the original application).

Thus, taking the penetration tests into consideration for assessing the inventive step amounts to reformulate the original problem which is not permitted according to the jurisprudence of the EPO.

Following this line of argument, the problem would simply be the provision of an alternative ophthalmic antibiotic and it would be obvious to use moxifloxacin by turning to D1 which describes the newest generation of quinolone antibiotics (compared to D3) and which specifically singles out moxifloxacin (in claim 2).

4.3. The opposition division's opinion is different.

The problem in the original application is mentioned as providing "improved compositions (...) of antibiotics that are _more effective_ than existing antibiotics against key ophthalmic pathogens..." (p. 2, l. 4,3 of the original application). The property which is sought is a better efficacy. The efficacy of an antibiotic certainly includes its pure antibacterial activity, expressed by the minimum inhibitory concentration (MIC) and illustrated by the table on p. 6 (of the original application), but it also inherently includes different pharmacokinetic properties, among which the ability to reach the microorganism in the infected tissue, i.e. the penetration properties. Indeed, an antibiotic having a very low MIC but very poor penetration properties would be useless.

Therefore the opposition division considers that the problem of enhancing the penetration properties of an antibiotic is implicitly included in the more general problem of enhancing its efficacy, as formulated in the original application.

Thus the tests provided during the examination procedure (D11) are taken into consideration following the line of the EPO Guidelines (C, IV.9.11).

4.4. Accordingly, the technical problem can be formulated as the provision of a novel ophthalmic composition comprising a quinolone antibiotic having an enhanced efficacy

with respect to the ophthalmic antibiotics of D3, and this encompasses the ability to penetrate into the eye tissues.

The proposed solution is to use moxifloxacin which does solve this problem (see D11). There is no incentive in the prior art, neither in D3 which does not even mention moxifloxacin, nor in any other document to use moxifloxacin rather than any other quinolone antibiotic in order to get an ophthalmic antibiotic having a better efficacy than those cited in D3.

Thus the subject-matter of claims 1 to 24 meets the requirements for inventive step (A. 56).

5.   Since the grounds for opposition do not prejudice the maintenance of the patent as granted, the opposition is rejected pursuant to A. 102(2).

Entscheidungsgründe (Anlage)	Grounds for the decision (Annex)	Motifs de la décision (Annexe)

Datum
Date 30.10.2006
Date

Blatt
Sheet 6
Feuille

Anmelde-Nr.:
Application No.: 99 956 504.7
Demande n°:

Annex I:

D1: US 5 607 942
D2: US 4 990 517
D3: WO 90 01933
D4: WO 96 39146
D5: Elies W., Chemotherapie Journal, 7/3, 1998, 93-97
D6: Current Medical Research and Opinion, 21 (1), 2005, 93-94
D7: Ophthalmology, 112(3), 2005, 466-469
D8: Robertson et al., Penetration and distribution of moxifloxacin and ofloxacin into ocular tissues and plasma following topical ocular administration to pigmented rabbits
D9: Declaration of David W. Stroman
D10: Survey of ophthalmology, 50(Sup. 1), 2005, S32-S45
D11: Letter of reply made in the examination procedure and dated 28.10.2002
D12: Arch. Ophthalmology, 123, 2005, 1282-1283
D13: Ophthalmology, 112(11), 2005, 1992-1996
D14: Ophthalmology, 113(6), 2006, 955-959
D15: Survey of ophthalmology, 50(Sup. 1), 2005, S55-S63
D16: Ophthalmic fluoroquinolones market share
D17: Package insert of Vigamox
D18: Antimicrobial agents and chemotherapy, 41(1), 1997, 101-106

Article 106
Decisions subject to appeal

(1) An appeal shall lie from decisions of the Receiving Section, Examining Divisions, Opposition Divisions and the Legal Division. It shall have suspensive effect.

(2) An appeal may be filed against the decisions of the Opposition Division even if the European patent has been surrendered or has lapsed for all the designated States.

(3) A decision which does not terminate proceedings as regards one of the parties can only be appealed together with the final decision, unless the decision allows separate appeal

(4) The apportionment of costs of opposition proceedings cannot be the sole subject of an appeal.

(5) A decision fixing the amount of costs of opposition proceedings cannot be appealed unless the amount is in excess of that laid down in the Rules relating to Fees.

Article 107
Persons entitled to appeal and to be parties to appeal proceedings

Any party to proceedings adversely affected by a decision may appeal. Any other parties to the proceedings shall be parties to the appeal proceedings as of right.

Article 108
Time limit and form of appeal

Notice of appeal must be filed in writing at the European Patent Office within **two months** after the date of notification of the decision appealed from. The notice shall not be deemed to have been filed until after the fee for appeal has been paid. Within **four months** after the date of notification of the decision, a written statement setting out the grounds of appeal must be filed.

Further information concerning the filing of an appeal

(a) The appeal is to be filed with the European Patent Office either at its seat in Munich, at its branch at The Hague or at its Berlin sub-office. The postal addresses are as follows:

(i) European Patent Office D-80298 Munich Germany (Telex: 523656 epmu d) (Fax: +49 89 2399-4465)	(ii) European Patent Office Branch at The Hague Patentlaan 2 Postbus 5818 NL-2280 HV Rijswijk (ZH) Netherlands (Telex: 31651 epo nl) (Fax: +31 70 340-3016)	(iii) European Patent Office Berlin sub-office D-10958 Berlin Germany (Fax: +49 30 25901-840)

(b) The notice of appeal must contain the name and address of the appellant in accordance with the provisions of Rule 26(2)(c) EPC, and a **statement** identifying the decision which is impugned and the extent to which amendment or cancellation of the decision is requested (see Rule 64 EPC). The notice of appeal and any subsequent submissions stating the grounds for appeal must be signed.

(c) Notice of appeal must be **filed in writing** (typewritten or printed (Rule 36(2) EPC), by telegram, telex or fax (Rule 36(5) EPC; OJ EPO 6/89, 219-225; OJ EPO 9/89, 396)).

(d) The fee for appeal is laid down in the Rules relating to Fees. The equivalents in the national currencies in which the fee for appeal can be paid are regularly published in the Official Journal of the European Patent Office under the heading "Guidance for the payment of fees, costs and prices".

In re Application of: Cagle, et al.

Serial No.: 10/715,055

Confirmation No.: 3314

Filed: November 17, 2003

Examiner: Fay, Zohreh A.

Group Art Unit: 1614

For: METHOD OF TREATING OPHTHALMIC INFECTIONS WITH
      MOXIFLOXACIN COMPOSITIONS

DECLARATION OF DAVID W. STROMAN, Ph.D.
UNDER C.F.R.§1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1.   I, David W. Stroman, declare as follows:

2.   I am the Director of Anti-Infective Microbiology for Alcon Research, Ltd.
("Alcon"). I have been employed in this capacity since August of 1990. Prior to
that date, I was affiliated with various research efforts in the biomedical field,
including infectious disease research. I was awarded a Ph.D. in Biochemistry
and Molecular Biology by the University of Oklahoma Medical School in 1970
and performed postdoctoral studies in the Department of Microbiology and
Immunology of Washington University School of Medicine from 1970 to 1972.
Further details regarding my educational background and experience in the field
of biomedical research are provided in my Curriculum Vitae, a copy of which is
attached as Appendix A.

3.   I have been responsible for all of Alcon's research programs in the field of ophthalmic anti-infective products since 1990. As a result of this experience, I have become very familiar with the state-of-the-art relative to the prevention and treatment of ophthalmic infections, particularly infections caused by pathogenic bacteria and viruses.

4.   The microorganisms that cause disease in humans (i.e., "pathogens") vary from one part of the body to another. Consequently, the bacteria that are considered to be pathogens relative to the eye may not be pathogens in other parts of the body and *vice versa*. For example, *Staphylococcus epidermidis* is a major pathogen in endophthalmitis, which is a serious, sight-threatening ocular infection, but is part of the normal flora on human skin. Another pathogen that can lead to serious ocular infections (as well as other infections) is *Pseudomonas aeruginosa*, which is a Gram negative bacterium. Ocular infections involving *Pseudomonas aeruginosa*, if left untreated, may cause a patient to lose their vision and perhaps even the affected eye itself.

5.   I am a co-inventor of the invention claimed in the pending United States patent application captioned above (the "pending application"). The invention resulted from a research program at Alcon that was directed to the discovery of new ocular anti-infective compositions that would be more efficacious than Alcon's existing product line in the mid-1990's. That product line included TOBREX®, which contains tobramycin, an aminoglycoside antibiotic, and CILOXAN®, which contains ciprofloxacin, a second generation fluoroquinolone antibiotic. My objectives in directing this research program were to identify new anti-infective agents that had: (a) a broad spectrum of activity against ocular pathogens, particularly with respect to isolates of ocular pathogens that had developed resistance to the fluoroquinolones utilized in existing ocular anti-infective products (e.g., ciprofloxacin); (b) greater potency than either tobramycin or ciprofloxacin; and (c) superior ocular bioavailability, relative to ciprofloxacin and ofloxacin, which represented the state-of-the-art in ocular fluoroquinolone anti-infective therapy prior to our invention.

6.   Alcon evaluated many compounds, including fluoroquinolones, before selecting moxifloxacin for its next generation ocular anti-infective product. As discussed below, this selection was based on unique properties of formulated moxifloxacin, particularly its superior ocular bioavailability.

7.   I have reviewed the following materials in connection with the preparation of this Declaration: (a) the Office Action from the United States Patent and Trademark Office on the pending application bearing a mail date of April 4, 2007 (the "Office Action"); and (b) U.S. Patent No. 5,607,942 (Petersen, et al), WO 90/01933 (Cagle, et al.) and U.S. Patent No. 5,597,560 (Bergamini, et al.). It is my understanding that the Examiner is asserting that the invention claimed in the pending application is not patentable in view of these prior publications. For the

reasons expressed below, I believe that the invention claimed in the pending application is clearly not obvious in view of the foregoing references.

8.　　The ocular bioavailability of moxifloxacin following topical application of an ophthalmic moxifloxacin composition to the eye is superior to that of other fluoroquinolones. As discussed below, the superior ocular bioavailability of moxifloxacin, when administered via an ophthalmic composition, has been demonstrated via numerous scientific studies. Such studies have been conducted both by Alcon scientists and others engaged in ophthalmic anti-infective research.

9.　　In column 58 of the '942 patent, *in vitro* data (i.e., minimum inhibitory concentration or "MIC" values) for 18 fluoroquinolone compounds is presented. The MIC values for the 18 compounds are compared to the MIC values for the second generation fluoroquinolone compound ciprofloxacin. The data presented in column 58 of the '942 patent are of relatively little value to a person searching for improved ocular anti-infective therapies for at least the following reasons:

　　(a) The spectrum of bacteria utilized in the testing is not the same as that typically utilized to evaluate anti-infective agents for possible use in preventing or treating ophthalmic infections. In particular, there is no data for *Pseudomonas aeruginosa*, which is the most dangerous ocular pathogen, relative to infections that may cause a loss of sight.

　　(b) There is also no data for bacteria identified as quinolone-resistant, i.e., bacteria that have developed resistance to prior quinolones used in ophthalmology, particularly ciprofloxacin and ofloxacin, such as quinolone resistant *Staphylococcus* species. Consequently, it is not possible to determine if the compounds identified in the '942 patent would be any more effective against such bacteria than existing fluoroquinolones, such as ciprofloxacin and ofloxacin.

　　(c) There is no MIC data in the '942 patent relative to moxifloxacin.

　　Thus, when I read the '942 patent, I would not consider that one of the compounds disclosed in this document is particularly useful for treating ophthalmic infections, nor would I assume that moxifloxacin has better properties than the other compounds disclosed in this document. In any event, there is no data in the '942 patent from which the efficacy of moxifloxacin in the treatment of ophthalmic infections via topical application to the eye might be predicted.

10.　　Although the '942 patent does not provide any insight relative to the possible efficacy of moxifloxacin in the treatment of ophthalmic infections, an article by J.M. Woodcock et al., published in the scientific journal <u>Antimicrobial Agents and Chemotherapy</u> (American Society of Microbiology) in January of

1997, did provide relevant information. A copy of the Woodcock et al. article is attached as Appendix B.

11.   As indicated in the abstract on page 1 of the Woodcock et al. article, the authors found that moxifloxacin, which is referred to in the article by means of the code number "BAY 12-8039", was less active against *Pseudomonas aeruginosa* than ciprofloxacin. In fact, the data presented in Table 1 on page 102 of the article indicate that moxifloxacin is 2 to 8 fold less active against *Pseudomonas aeruginosa* than ciprofloxacin, depending on the MIC level tested, i.e., 50% or 90%. (There was an 8 fold difference at the 50% value, i.e., 2 µg/ml for moxifloxacin versus 0.25 µg/ml for ciprofloxacin, and a 2 fold difference at the 90% value, i.e., 8 µg/ml versus 4 µg/ml).

12.   Based on the data presented in the Woodcock et al. article, a scientist familiar with the requirements for ophthalmic anti-infective products could not have predicted that moxifloxacin would be superior to ciprofloxacin and other fluoroquinolones as an ocular anti-infective agent, because it exhibited relatively poor activity against one of the bacterium that is of greatest concern relative to sight-threatening ophthalmic infections. In fact, I was aware of the findings of Woodcock, et al. regarding the poor *in vitro* activity of moxifloxacin against *Pseudomonas aeruginosa*, compared to ciprofloxacin, and this inferiority was a major concern. Surprisingly, however, the superior ocular penetration properties of moxifloxacin *in vivo* more than compensated for the limited *in vitro* activity of this compound against *Pseudomonas aeruginosa*.

13.   Contrary to the prediction that might have been made based on the Woodcock, et al. article, Alcon's scientists discovered that the overall potency and penetration of our ophthalmic moxifloxacin compositions is, in fact, much greater than that of formulations containing ciprofloxacin, ofloxacin and other quinolones previously utilized to treat ophthalmic infections. This superiority is largely due to the ability of the compound in solution to penetrate the cornea, which is clearly not mentioned or implied in the '942 patent.

14.   In one of Alcon's initial evaluations of ophthalmic compositions containing moxifloxacin, we utilized compositions containing moxifloxacin at concentrations of 0.2, 0.3 and 0.5% (wt. %) to evaluate the efficacy of ocular moxifloxacin compositions in the treatment of keratitis infections attributable to *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Keratitis is an infection involving the intrastromal layer of the corneal tissues. In order to treat this condition effectively, the anti-infective agents utilized must be capable of penetrating into the cornea. An animal model here been developed to evaluate anti-infective agents relative to this objective. See Hobden, et al., "*Pseudomonas aeruginosa* keratitis in leukopenic rabbits", Current Eye Research, volume 12, pages 461-467 (1993); a copy is attached as Appendix C. In early 1999, a study based on this model was conducted by one of the authors of the above-identified article, Richard O'Callaghan. The results of the study are provided in the attached Technical

Report (Appendix D). The results of this study confirmed that ophthalmic compositions containing moxifloxacin at concentrations of 0.2, 0.3 and 0.5%, respectively, produce a level of drug in the corneal tissues that is efficacious. Moreover, it was determined that although the *in vitro* activity of moxifloxacin against *Pseudomonas aeruginosa* is 8 fold <u>less</u> than the activity of ciprofloxacin, the 0.3% moxifloxacin solution utilized in this evaluation produced results equivalent to those seen in our prior testing of a 0.3% ciprofloxacin solution (i.e., CILOXAN® Solution) in the same model. The ability of the 0.3% moxifloxacin solution to match the results obtained with a ciprofloxacin solution, despite the inferior *in vitro* activity of moxifloxacin against *Pseudomonas aeruginosa*, is the result of the superior corneal penetration achieved with the moxifloxacin compositions. This evidence of superior ocular penetration was confirmed in subsequent studies conducted by various investigators. Those studies are discussed below.

15.   The pharmacokinetics of moxifloxacin relative to several other fluoroquinolones has been extensively studied by my colleagues at Alcon, as well as other scientists and physicians.   The findings of numerous scientists regarding the properties of moxifloxacin and other fluoroquinolones are discussed in a series of scientific articles published as a Special Supplement to the November, 2005 edition of <u>Survey of Ophthalmology</u>, International Review Journal (volume 50, supplement 1). A copy of this publication is attached as Appendix E. The article by Robertson, et al., which begins on page S32 of the publication, is specifically directed to a survey of studies relating to the pharmacokinetic properties of ophthalmic moxifloxacin formulations and other ophthalmic fluoroquinolone formulations.

16.   The data presented in the attached article by Robertson, et al. (Appendix E, pages S32-S45) demonstrate the superior ocular bioavailability of moxifloxacin, relative to other fluoroquinolones. For example, the corneal permeability data presented in Table 3 (page S36) show that moxifloxacin penetrates the cornea much more readily than other fluoroquinolones. The ability of moxifloxacin to penetrate the cornea following application via an aqueous solution enables this drug to reach intraocular tissues in amounts sufficient to treat or prevent infections in those tissues. As shown in Table 4 (page S37), moxifloxacin reaches the intraocular fluid (i.e., aqueous humor) and intraocular tissues (e.g., iris-ciliary body) at much higher levels than ofloxacin. Similar results are shown in Table 6 (page S39) and Table 10 (page S43). The superior ocular bioavailability of moxifloxacin in solution is a surprising finding that is not predicted by the Petersen, et al., Cagle, et al and Bergamini, et al. publications cited by the Examiner in the Office Action.

17.   The experiments described in the attached Robertson, et al. article (Appendix E, pages S-43-S45 involved testing of ophthalmic solutions containing moxifloxacin at concentrations of 0.3 and 0.5%. The broadest claim of the pending application specifies a concentration range of 0.1 to 1%. The superior

ocular penetration properties of moxifloxacin are not dependent on the use of a particular concentration within this range (e.g., 0.3% or 0.5%). Rather, as explained in greater detail below, these properties are prevalent over the entire range of 0.1 to 1%.

18.   The bioavailability of drugs when administered via topical application to the eye can be evaluated by various ocular penetration models. Such models have proven to be quite reliable in simulating actual *in vivo* drug levels. One such model is described in Schoenwald, et al., "Corneal Penetration Behavior of ß-Blocking Agents I", <u>Journal of Pharmaceutical Sciences</u>, volume 72, no. 11, pages 1266-1272, November 1983; a copy is attached as Appendix F. This model measures the rate of diffusion of a drug across the cornea. It was utilized to evaluate the ocular penetration properties of moxifloxacin upon application of ophthalmic compositions containing this compound at concentrations of 0.1, 0.3, 0.5, 0.75 and 1.0%, respectively. An aqueous vehicle corresponding to the vehicle used in Alcon's VIGAMOX® (0.5% moxifloxacin) Ophthalmic Solution was utilized for these compositions.[*] The results of this experiment were as follows:

Drug Concentration	Diffusion Rate (Micrograms/ Minute)	Total Amount of Drug Penetration at 240 Minutes in Micrograms
0.1%	0.86	180
0.3%	2.16	455
0.5%	3.20	667
0.75%	4.54	930
1.0%	6.00	1,197

A graph and table showing the relative diffusion rates, as well as the total amount of moxifloxacin which penetrated the cornea following application of the respective compositions, are attached as Appendices G1 and G2, respectively. The results show that increasing the concentration of moxifloxacin increases the rate of diffusion of moxifloxacin across the cornea, as well as the amount of moxifloxacin that accumulates on the endothelial (aqueous humor) side of the cornea. As shown in Appendix G1, the relationship between moxifloxacin concentration and ocular penetration is linear over the concentration range of 0.1 to 1.0%.

19.   The *in vitro* corneal penetration model discussed in paragraph 18 above has been utilized to compare the ocular penetration of ophthalmic solutions containing moxifloxacin and gatifloxacin, respectively. It was found that the corneal penetration of moxifloxacin is about 3.6 times greater than that of

---

[*] The VIGAMOX® vehicle contains boric acid, sodium chloride and water, and has a pH of 6.8.

gatifloxacin. This finding is described in the following publication: Owen, et al. "Corneal Penetration and Changes in Corneal Permeability of Moxifloxacin versus Gatifloxacin", Investigative Ophthalmology Visual Science 45: E-Abstract 4910, 2004; a copy is attached as Appendix H. The relative ocular penetration properties of moxifloxacin and gatifloxacin have also been evaluated via *in vivo* testing, as described in the following publication: Kim, et al., "Aqueous Penetration and Biological Activity of Moxifloxacin 0.5% Ophthalmic Solution and Gatifloxacin 0.3% Solution in Cataract Surgery Patients", Ophthalmology, volume 112, issue 11, pages 1992-1996, November 2005; a copy is attached as Appendix I. The *in vivo* study described in the foregoing article revealed that the aqueous humor level of moxifloxacin following topical application of a 0.5% moxifloxacin solution was 3.8 times greater than the aqueous humor level of gatifloxacin following application of a 0.3% gatifloxacin solution. Thus, in both the *in vitro* corneal penetration model and in the *in vivo* studies, moxifloxacin penetrated the cornea about 3.6 to 3.8 times better than gatifloxacin. This correlation between the results in the *in vitro* model and those seen in the *in vivo* testing demonstrates the reliability of the *in vitro* model as a tool for predicting the *in vivo* ocular penetration of fluoroquinolones, such as moxifloxacin. The *in vitro* data discussed in paragraph 18 above is therefore believed to provide a very realistic representation of the ocular penetration properties in patients that are treated with the ophthalmic compositions of our invention containing 0.1 to 1.0 % moxifloxacin.

20. In summary, the superior ocular bioavailability properties of moxifloxacin, when administered topically via the compositions of the present invention, has been demonstrated via numerous studies conducted by Alcon's scientists and others engaged in the field of ophthalmic anti-infective research. This superiority applies to both prior second generation fluoroquinolones, such as ciprofloxacin and ofloxacin, and fourth generation fluoroquinolones, such as gatifloxacin. These superior ocular bioavailability properties are not suggested in any manner by the Petersen, et al. reference or other references cited in the Office Action, nor any other prior publications of which I am aware. The properties are therefore truly unexpected. The experimental testing discussed in paragraph 18 above shows that the ocular penetration properties of moxifloxacin are linear across the entire 0.1 to 1.0 % concentrations utilized in the compositions and methods of treatment of our invention. The *in vitro* testing methodology described in paragraph 18 has been demonstrated to be a reliable model for the *in vivo* bioavailability of ophthalmic anti-infective agents. Based on these findings, as well as the numerous comparative tests of ophthalmic compositions containing moxifloxacin discussed in Appendix E, it can be reasonably concluded that the unexpected penetration obtained with our invention occurs at all concentrations across the range of 0.1 to 1.0 %.

21.   The unexpected results achieved with our invention have greatly contributed to its commercial success. Alcon Laboratories, Inc. introduced a new anti-infective product based on our invention, i.e., VIGAMOX® (0.5% moxifloxacin) Ophthalmic Solution in 2003. The acceptance of VIGAMOX® in the medical community has been very rapid. This product was launched in the U.S. in 2003, and achieved total annual global sales of more than $100,000,000 in 2004. The acceptance of this product has continued to grow dramatically, with global sales in 2005 of about $146,000,000 and global sales in 2006 of about $185,000,000.

22.   I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Declared at: Fort Worth, Texas, USA, on this _22 ND_ day of May, 2007.

David W. Stroman
David W. Stroman, Ph.D.

Attachments: Appendix A
                    Appendix B
                    Appendix C
                    Appendix D
                    Appendix E
                    Appendix F
                    Appendix G1 and G2
                    Appendix H
                    Appendix I

CURRICULUM VITAE

Name: David W. Stroman

Place and Date of Birth: Corpus Christi, TX - June 1, 1944

Home Address: 7214 Native Oak Lane
Irving, TX 75063

Home telephone - (972) 401-9627
Home e-mail – DWStroman@AOL.com

Work telephone - (817-551-4354)
Work FAX number – (817-551-9933)
Work e-mail – david.stroman@alconlabs.com

Education:

1962-1966   B.S., Chemistry, Southern Nazarene University, Bethany, OK

1966-1970   Ph.D., Biochemistry and Molecular Biology, University of
Oklahoma Medical School, Oklahoma City, OK
Thesis: Expression of the clustered arginine genes of *E. coli*

1970-1972   Postdoctoral studies, Department of Microbiology and
Immunology, Washington University School of Medicine, St.
Louis, MO

1974   4th International Training Course in Membrane Biophysics,
Yale University School of Medicine, New Haven, CN

Professional Experience:

8/90 to present Director, Anti-Infective Microbiology, Alcon Research, Ltd.,
Ft. Worth, TX

6/88-7/90   President, Bissendorf Biosciences Inc., Richardson, TX

6/87-6/88   Manager, Biotechnology Ventures, Phillips Petroleum
Company

10/85-6/88   Vice President, Phillips 66 Biosciences Corporation

Formed and obtained financing for three "biotech" companies
as a part of Phillips overall biopharmaceutical strategy.

Served on the Board of Directors for the three companies:

Appendix A

-Biociences Corporation of Texas, Houston, TX
    (J/V with Baylor College of Medicine)
-Bissendorf Biosciences, GmbH, Hannover, W. Germany
    (J/V with Bissendorf Peptide, GmbH)
-Wadley Biosciences, Ltd., Dallas, TX
    (J/V with Wadley Institutes of Molecular Medicine)

8/85-6/87	Coordinator for Biotechnology Licensing, Patent and Licensing Division, Phillips Petroleum Company
12/81-1/86	Scientific liaison between Phillips Petroleum and SIBIA (Phillips's J/V with The Salk Institute) with technical responsibility for contract research
12/84-8/85	Section Supervisor, Recombinant DNA Product Research, Biotechnology Division, Phillips Petroleum Company
7/81-12/84	Research Molecular Biologist and Group Leader, Biotechnology Division, Phillips Petroleum Company, Bartlesville, OK
9/76-12/76	Visiting Instructor, Department of Biology, Kalamazoo College, Kalamazoo, MI
12/72-7/81	Research Scientist, Infectious Diseases Research, The Upjohn Company, Kalamazoo, MI
9/70-11/72	NIH (NCI) Postdoctoral Fellow, Department of Microbiology and Immunology, Washington University School of Medicine, St. Louis, MO
6/67-8/70	Research Assistant, Department of Biochemistry and Molecular Biology, University of Oklahoma Medical School
9/66-5/67	Teaching Assistant, Department of Biochemistry and Molecular Biology, University of Oklahoma Medical School, Oklahoma City, OK
9/64-8/66	Research Assistant, Department of Chemistry, Southern Nazarene University
9/64-5/65 9/65-7/66	Teaching Assistant, Department of Chemistry, Southern Nazarene University, Bethany, OK

Publications-*Posters-Talks*:

Stroman DW, Dajcs JJ, Vidal R, Mridvika, Martinez C, Thibodeaux BA, O'Callaghan RJ, Schlech BA: Combination of pharmacokinetics and susceptibility data as a predictive indicator for prevention of bacterial keratitis. Ocular Microbiology and Immunology Group (OMIG) Meeting 2004

*Dajcs JJ, Macke LM, Clark LL, Karr DL, Bartell JG, **Stroman DW**: Molecular characterization of novel coagulase-negative* Staphylococcus *clinical isolates. ICAAC Annual Meeting 44:2004*

Stroman DW, Cupp GA, Clark LL, Macke LM, Bartell JG, McLean CH, Karr DL, Shannon SP, Schlech BA: Conjunctivitis, bacterial resistance, therapy success and failure. Australian Society of Microbiology Meeting 2004

Roland P, Stroman DW, Parry D: Microbiology of acute otitis media with a tympanostomy tube. American Academy of Otolaryngology – Head and neck Surgery Annual Meeting 2004

Bartell JB, Karr D, Clark L, McLean C, Macke L, Mendoza B, Stroman DW: Characterization of Streptococcus parasanguinis *isolates associated with otic and ocular infections. ASM Annual Meeting 2004*

*Roland PS, Kreisler LS, Reese B, Anon JB, Lanier B, Conroy PJ, Wall GM, Dupre SJ, Potts S, Hogg G, **Stroman DW**, McLean C: Topical ciprofloxacion/dexamethasone otic suspension is superior to ofloxacin otic solution in the treatment of children with acute otitis media with otorrhea through tympanostomy tubes. Pediatrics 113: 40-6, 2004*

Stroman DW, Schlech BA, Alfonso E, Wilhelmus K, Abshire R: The treat of atypical Mycobacterium *in ophthalmology. Ocular Microbiology and Immunology Group (OMIG) Meeting 2003*

*Bartell JB, Karr D, Clark L, McLean C, Macke L, Mendoza B, Cupp G, **Stroman DW**: Molecular characterization of a clinically important ampicillin resistant subpopulation of* Haemophilus influenzae. *ICAAC Annual Meeting 43: 2003*

Stroman DW, Mendoza B, Sukplang P, Berry R, Schlech BA: Kinetics of killing of ocular isolates of Staphylococcus aureus *and* Staphylococcus epidermidis *by moxifloxacin. ARVO Annual Meeting 2003:1463, 2003*

Katz HR, Andrews W, Creager D, deLeon J, Merkley K, Gower L, Stroman DW, Nicholson N, Potts S, Moxifloxacin Study Group: Moxifloxacin

ophthalmic solution 0.5% hastens cure and eradicates the causative pathogens of bacterial conjunctivitis in pediatric and adult patients. ARVO Annual Meeting 2003:2114, 2003

Schlech BA, **Stroman DW**, Gower L, Cupp G: *Eradication of bacteria from infected eyes by a three day BID treatment with moxifloxacin ophthalmic solution 0.5%. ARVO Annual Meeting 2003:2116, 2003*

Stroman DW, Mendoza, B, Sukplang P, Schlech BA: *In vitro kinetics of kill of Gram-positive bacteria by commercial ophthalmic fluoroquinolones. ASCRS Annual Meeting 2003*

Roland PS, Anon JB, Moe RD, Conroy PJ, Wall GM, Dupre SJ, Krueger KA, Potts S, Hogg G, **Stroman DW**: Topical ciprofloxacion/dexamethasone is superior to ciprofloxacin alone in pediatric patients with acute otitis media and otorrhea through tympanostomy tubes. Laryngoscope 113:2116-22, 2003

Stroman DW: Update on methods of assessing microbiologic success or failure in patients with otic disease. Ear Nose Throat J 82 (Suppl 2): 14-7, 2003

Stroman DW, Clark L, Mclean C, Mendoza B, Bartell JG: *Molecular characterization of clinical isolates identified phenotypically as Enterobacter cloacae. ICAAC Annual Meeting 42:145, 2002*

Bartell JG, Clark l, Macke L, **Stroman DW**: *Enterobacter species isolated from community-acquired acute otitis externa and conjunctivitis. ICAAC Annual Meeting 42:366, 2002*

Mclean CH, Clark LL, Bartell JG, **Stroman DW**: *Novel* Pseudomonas *species recovered from acute otitis externa. ASM Annual Meeting 102:169, 2002*

Dajcs JJ, Thibodeaux BA, Girgis DO, Traidej M, O'Callaghan RJ, Stroman DW, Schlech BA, Carreras N, Vallet JA: *The effectiveness of fluoroquinolone antibiotics administered prophylactically for Staphylococcus aureus keratitis. ARVO Annual Meeting 2002:1583, 2002*

Stroman DW, Cupp GA, Katz HR, Do D: *Characterization and antibiotic susceptibility of bacteria from healthy eyes in the US and India. ARVO Annual Meeting 2002:1584, 2002*

Kane ST, Cochran DC, **Stroman DW**, McLean CH, Callegan MC: *Genotypic and phenotypic analysis of putative virulence factors associated with*

Bacillus *ocular infection isolates. ARVO Annual Meeting 2002:1598, 2002*

Roland PS, **Stroman DW**: Microbiology of acute otitis externa. Laryngoscope 112:1166-77, 2002

Moreau JM, Conerly LL, Hume EB, Dajcs JJ, Girgis DO, Cannon BM, Thibodeaux BA, **Stroman DW**, O'Callaghan RJ: Effectiveness of mupirocin and polymyxin B in experimental *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Serratia marcescens* keratitis. Cornea 21:807-11, 2002

Stroman DW, Mclean C, Clark L, Cupp G, Schlech BA, Bartell J: Characterization of Acinetobacter *isolates from acute otitis externa. ASM Annual Meeting 101:682-3, 2001*

Stroman DW, Clark L, Macke L, Mendoza B, Schlech BA, O'Brien T: Moxifloxacin activity against quinolone resistant Staphylococcal ocular isolates. ARVO Annual Meeting Abs S255, 2001

Moreau JM, Dajcs JJ, Stroman DW, Schlech BA, Ke TL, Thibodeaux BA, Marquart ME, O'Callaghan RJ: A rabbit model of Staphylococcus *keratitis for measuring the effectiveness of prophylactic antibiotics. ARVO Annual Meeting Abs S740, 2001*

Stroman DW, Roland PS, Dohar J, Burt W: Microbiology of normal external auditory canal. Laryngoscope 111:2054-9, 2001

Dajcs JJ, Moreau JM, Thibodeaux BA, Traidej M, Austin MS, Marquart ME, **Stroman DW**, O'Callaghan RJ: Effectiveness of ciprofloxacin and ofloxacin in a prophylaxis model of *Staphylococcus* keratitis. Cornea 20:878-80, 2001

Dajcs JJ, Moreau JM, **Stroman DW**, Schlech BA, Ke TL, Thibodeaux BA, Girgis DO, Caballero AR, O'Callaghan RJ: The effectiveness of tobramycin and OCUFLOX in a prophylaxis model of *Staphylococcus* keratitis. Curr Eye Res 23:60-3, 2001

Stroman DW, Mclean C, Clark L, Cupp G, McDonald M, Schlech BA: Coryneform bacteria from healthy and diseased ears in 1998-2000. ICAAC Annual Meeting 40:445, 2000

Block SL, Hedrick J, Tyler R, Smith A, Findlay R, Keegan E, **Stroman DW**: Increasing bacterial resistance in pediatric acute conjunctivitis (1997-1998). Antimicrob Agents Chemother 44:1650-4, 2000

Stroman DW, Cupp GA, Clark LL, Mclean CH, McDonald MM, Abshire R, Block S: New Haemophilus species in pediatric conjunctivitis patients resistant to ampicillin. Ocular Microbiology and Immunology Group (OMIG) Meeting 1999

Stroman DW, Mclean C, McDonald M, Clark L, Cupp G, Runner J: Coagulase-negative staphylococci isolated from acute otitis externa. ASM Annual Meeting 99:251, 1999

Hume EB, Conerly LL, Moreau JM, Cannon BM, Engel LS, **Stroman DW**, Hill JM, O'Callaghan RJ: Serratia marcescens keratitis: strain-specific corneal pathogenesis in rabbits. Curr Eye Res 19:525-32, 1999

Block SL, Hedrick J, Stroman DW, Tyler RD, Smith RA, Findlay R, Keegan E: Bacterial pathogens of pediatric conjunctivitis (1997-1998). ICAAC (Interscience Conference on Antimicrobial Agents and Chemotherapy) Annual Meeting 38:89, 1998

Stroman DW, McLean C, Rogers J: Discrimination between B. anthracis, B. cereus, B. mycoides and B. thuringiensis strains by automated rRNA operon ribotyping. 3rd International Conference on Anthrax, Plymouth, UK 1998

McLean C, Stroman DW: Discrimination between Bacillus cereus and B. thuringiensis by automated ribosomal operon ribotyping. ASM (American Society of Microbiology) Annual Meeting 98:152, 1998

Conerly LL, Moreau JM, Engel LS, Stroman DW, Hill JM, O'Callaghan RJ: Rabbit model of Serratia keratitis: growth and ocular pathology. ARVO (Association for Research in Vision and Ophthalmology) Annual Meeting Abs S872, 1997

Hagenson MJ, Holden KA, Parker KA, Wood PJ, Cruze JA, Fuke M, Hopkins TR, **Stroman DW**: Expression of streptokinase in Pichia pastoris yeast. Enzyme Microb Technol 11:650-656, 1989

Stroman DW, Hagenson MJ: Methylotrophic yeast as vehicles for heterologous gene expression. Dechema Monographs - VCH Verlagsgesellschaft 105:141-146, 1987

Brodasky TF, **Stroman DW**, Dietz A, Mizsak S: U56,407, a new antibiotic related to asukamycin: isolation and characterization. J Antibio (Tokyo) 36:950-6, 1983

Coats JH, **Stroman DW**: Recent studies in Streptomyces archromogenes subsp. rubradiris genetics. Proc. Intern. Colloquium at the Forschungs Institut

Borstel, September 29-October 1, 1976, E. Freerksen, I. Tarnok and J. H. Thumin (eds.), Gustav Fischer Verlag, Stuttgart, pp. 39-46, 1978

Dolak LA, Sokolski WT, Mizsak S, **Stroman DW**, Sebek OK: Microbial formation of 4-thiouracil. Antimicrob Agents Chemother 11:569-570, 1977

Stroman DW: Expression of the clustered arginine genes in *E. coli.* Ph.D. Dissertation. Univ Okla Med School. 1970

US Patents:

Gerald D. Cagle, Robert L. Abshire, **David W. Stroman**, Celeste Mclean, Linda L. Clark, John M Yanni: Methods for treating otic and ophthalmic infections. US Patent No. 6740664; issued 2004.

Gerald D. Cagle, Robert L. Abshire, **David W. Stroman**, John M Yanni: Ophthalmic antibiotic compositions containing moxifloxacin. US Patent No. 6716830; issued 2004.

Gerald D. Cagle, Robert L. Abshire, **David W. Stroman**, Celeste H. Mclean, Linda L. Clark, John M Yanni: Compositions and methods of treating otic, ophthalmic and nasal infections. US Patent No. 6509327; issued 2003.

Gerald D. Cagle, Robert L. Abshire, **David W. Stroman**, Celeste H. Mclean, Linda L. Clark, John M Yanni: Compositions and methods of treating ophthalmic and otic, infections. US Patent No. 6440964; issued 2002.

Gerald D. Cagle, Robert L. Abshire, **David W. Stroman**, John M Yanni: Methods of treating ophthalmic, otic, and nasal infections and attendant inflammation. US Patent No. 6395746; issued 2002.

Mary J. Hagenson, Katherine A. Barr, **D. W. Stroman**, Frank H Gaertner, Michael M. Harpold, and Ronald D. Klein: *Pichia pastoris* linear plasmids and DNA fragments thereof. US Patent No. 5665600; issued 1997.

David W. Stroman, James M. Cregg, Micheal M. Harpold, George T. Sperl: Transformation of yeasts of the genus *Pichia*. US Patent No. 4,879,231; issued November 7, 1989.

David W. Stroman, Paul F. Brust, Steven B. Ellis, Thomas R. Gingeras, Micheal M. Harpold, Juerg F. Tschopp: Regulatory region for heterologous gene expression in yeast. US Patent No. 4,855,231; issued August 8, 1989.

David W. Stroman, Paul F. Brust, Steven B. Ellis, Thomas R. Gingeras, Micheal M. Harpold, and Juerg F. Tschopp: Methanol inducible genes obtained from *Pichia* and methods of use. US Patent No. 4,808,537; issued February 28, 1989.

Thomas F. Brodasky and **David W. Stroman**: Antibiotic compound U-56,407 and process for recovery thereof from a fermentation broth. US Patent No. 4,595,770; issued June 17, 1986.

Alexander D. Argoudelis and **David W. Stroman**. Process for producing lincomycin nucleotides. US Patent No. 4,464,466; issued August 7, 1984.

Alexander D. Argoudelis and **David W. Stroman**: Lincomycin nucleotides. US Patent No. 4,383,109; issued May 10, 1983.

Alexander D. Argoudelis and **David W. Stroman**: Process for treating malaria. US Patent No. 4,368,193; issued Jan. 11, 1983.

In Vitro Activity of BAY 12-8039, a New Fluoroquinolone

J. M. WOODCOCK, J. M. ANDREWS, F. J. BOSWELL, N. P. BRENWALD, AND R. WISE*

Department of Microbiology, City Hospital NHS Trust, Birmingham B18 7QH, United Kingdom

The in vitro activity of BAY 12-8039, a new fluoroquinolone, was studied in comparison with those of ciprofloxacin, trovafloxacin (CP 99,219), cefpodoxime, and amoxicillin-clavulanate against gram-negative, gram-positive, and anaerobic bacteria. Its activity against mycobacteria and chlamydia was also investigated. BAY 12-8039 was active against members of the family Enterobacteriaceae (MIC at which 90% of strains tested were inhibited [MIC$_{90}$] ≤ 1 μg/ml, except for Serratia spp. MIC$_{90}$ 2 μg/ml), Neisseria spp. (MIC$_{90}$8, 0.015 μg/ml), Haemophilus influenzae (MIC$_{90}$, 0.03 μg/ml), and Moraxella catarrhalis (MIC$_{90}$, 0.12 μg/ml), and these results were comparable to those obtained for ciprofloxacin and trovafloxacin. Against Pseudomonas aeruginosa, the quinolones were more active than the β-lactam agents but BAY-12-8039 was less active than ciprofloxacin. Strains of Stenotrophomonas maltophilia were fourfold more susceptible to BAY 12-8039 and trovafloxacin (MIC$_{90}$8, 2 μg/ml) than to ciprofloxacin. BAY 12-8039 was as active as trovafloxacin but more active than ciprofloxacin against Streptococcus pneumoniae (MIC$_{90}$, 0.25 μg/ml) and methicillin-susceptible Staphylococcus aureus (MIC$_{90}$8, 0.12 μg/ml). The activity of BAY 12-8039 against methicillin-resistant S. aureus (MIC$_{90}$, 2 μg/ml) was lower than that against methicillin-susceptible strains. BAY 12-8039 was active against anaerobes (MIC$_{90}$6 ≤ 2 μg/ml), being three- to fourfold more active against Bacteroides fragilis, Prevotella spp., and Clostridium difficile than ciprofloxacin. Against Mycobacterium tuberculosis, BAY 12-8039 exhibited activity comparable to that of rifampin (MICs ≤ 0.5 μg/ml). Against Chlamydia trachomatis and Chlamydia pneumoniae BAY 12-8039 was more active (MICs ≤ 0.12 μg/ml) than either ciprofloxacin or erythromycin and exhibited a greater lethal effect than either of these two agents. The protein binding of BAY 12-8039 was determined at 1 and 5 μg/ml as 30 and 26.4%, respectively. The presence of human serum (at 20 or 70%) had no marked effect on the in vitro activity of BAY 12-8039.

BAY 12-8039 is a new fluoroquinolone derivative with a chemical nomenclature of 1-cyclopropyl-7-[(S,S)-2,8-diazabicyclo[4.3.0]non-8-yl]-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-3-quinoline carboxylic acid. It shares structural similarities with other agents, namely, a cyclopropyl group at position 1 (as ciprofloxacin has), a methoxy group at position 8 (as AM155 has) (10), and a diazabicyclo group at position 7 (as BAY y3118 has) (5). Preliminary information suggests that BAY 12-8039 has enhanced activity against gram-positive bacterial pathogens (3). In this study, the activity of BAY 12-8039 was compared with that of other fluoroquinolones and the novel naphthyridone compound trovafloxacin (CP 99,219) (2) against a wide range of pathogens.

MATERIALS AND METHODS

Antimicrobial agents. The following agents were employed: BAY 12-8039 and ciprofloxacin (Bayer AG, Wuppertal, Germany), trovafloxacin (Pfizer Inc., Groton, Conn.), cefpodoxime (Roussel Uclaf, Romainville, France), amoxicillin and clavulanic acid (SmithKline Beecham, Worthing, United Kingdom), rifampin (Sigma, Poole, United Kingdom), rifampin (Sigma, Poole, United Kingdom), and erythromycin (Lilly Products, Basingstoke, United Kingdom). All agents were prepared and stored following the manufacturer's instructions.

Susceptibility testing. A total of 684 recent clinical isolates, 11 control strains, and 10 well-characterized β-lactamase-producing strains were tested. The control strains used were Escherichia coli NCTC 10418 and ATCC 25922, Pseudomonas aeruginosa NCTC 10662 and ATCC 27853, Staphylococcus aureus NCTC 6571 and ATCC 25923, Streptococcus pneumoniae NCTC 7465 and ATCC 49619, Haemophilus influenzae NCTC 11931 and ATCC 49247, and Enterococcus faecalis ATCC 29212. Susceptibilities were determined by a standard agar plate dilution method following recommendations in reference 1. Briefly, Iso-Sensitest agar (pH 7.2; Unipath, Basingstoke, United Kingdom) was employed for aerobic bacteria, supplemented with 50 μg of 1-(4-nitrophenyl)-glycerol (BDH, Poole, United Kingdom) per ml where necessary to prevent swarming. Supplements of 5% horse blood (Bradsure Biologicals, Loughborough, United Kingdom) and 20 μg of NAD (Sigma) per ml were added to support growth of fastidious bacteria.

For anaerobic bacteria, Wilkins-Chalgren agar (Unipath) supplemented with 50 μg of 1-(4-nitrophenyl)-glycerol per ml and 5% horse blood was used. All strains were tested at a final inoculum of 10^4 CFU and for a few selected strains at an increased inoculum of 10^6 CFU, using a multipoint inoculator (Denley Instruments, Billingshurst, United Kingdom). Plates were incubated at 35 to 37°C for 18 to 24 h in air; or, for fastidious bacteria, in an atmosphere enriched with 4 to 6% carbon dioxide; or, for anaerobic bacteria, in an anaerobic cabinet (Don Whitley, Shipley, United Kingdom) in an atmosphere of 10% hydrogen, 10% carbon dioxide, and 80% nitrogen.

The MIC was defined as the lowest antibiotic concentration at which no more than two colonies were observed. Amoxicillin and clavulanic acid were combined in a ratio of 2:1, and the results were recorded in terms of the amoxicillin MIC.

Mycobacterium susceptibility testing. The activity of BAY 12-8039 against mycobacteria was studied by an agar incorporation method using rifampin as a comparative agent. Recent clinical isolates of Mycobacterium tuberculosis (three resistant to one or more of the commonly used antimycobacterial agents and one susceptible strain) were studied. For both antibiotics a concentration range of 0.015 to 128 μg/ml (doubling dilutions up or down from 1 μg/ml) incorporated into Middlebrook 7H10 medium (Difco, Detroit, Mich.), containing 10% Middlebrook oleic acid-albumin-dextrose-catalase enrichment as a supplement, was used. Plates were incubated at 37°C in 5 to 10% carbon dioxide for 21 days. The lowest concentration of antibiotic that inhibited more than 99% of the bacterial population was considered to be the MIC (8).

Chlamydia susceptibility testing. The activity of BAY 12-8039 against one strain of Chlamydia pneumoniae and 3 strains of Chlamydia trachomatis was investigated in comparison with those of ciprofloxacin and erythromycin. The method employed was an adaptation of that of Webberley et al. (11). The MIC was taken as the lowest concentration to inhibit the development of inclusion bodies, and the minimum lethal concentration (MLC) was defined by the absence of inclusion bodies after a further 48-h incubation in drug-free medium.

Serum effect. The effect of human serum on the MIC and minimum bactericidal concentration (MBC) of BAY 12-8039 was determined for two strains each of Streptococcus pyogenes, S. pneumoniae, methicillin-sensitive S. aureus (MSSA), Moraxella catarrhalis, E. coli, and Klebsiella pneumoniae. A microdilution method was employed using Iso-Sensitest broth (Unipath) containing 20 or 70% human serum (Bradsure Biologicals) and supplemented for fastidious bacteria with 5% lysed horse blood and 20 μg of NAD per ml. Concentration ranges (doubling dilutions up or down from 1 μg/ml) of BAY 12-8039 were 0.008 to 8 μg/ml or 0.03 to 32 μg/ml (for fastidious bacteria). A final inoculum of 10^5 CFU/ml was used. Following incubation at 35 to 37°C in air or 4 to 6% carbon dioxide (for fastidious bacteria), 50 μl of broth culture was subcultured onto appropriate antibiotic-free medium for MBC determinations. The MIC was defined as the

* Corresponding author.

Appendix B

TABLE 1. The in vitro activity of BAY 12-8039 in comparison with those of other antimicrobial agents

Organism (no.)	Antibiotic	MIC (µg/ml)[a]		
		50%	90%	Range
E. coli (39)	BAY 12-8039	0.06	1	0.03–32
	Trovafloxacin	0.06	1	0.015->128
	Ciprofloxacin	0.015	0.5	0.008–64
	Cefpodoxime	0.25	4	0.12->128
	Amoxicillin-clavulanate	2	16	0.5–32
Klebsiella spp. (30)	BAY 12-8039	0.12	0.5	0.06–4
	Trovafloxacin	0.12	0.5	0.06–8
	Ciprofloxacin	0.03	0.25	0.015–4
	Cefpodoxime	0.25	8	0.12–64
	Amoxicillin-clavulanate	4	8	1–32
P. mirabilis (30)	BAY 12-8039	0.25	0.25	0.12–0.5
	Trovafloxacin	0.25	0.25	0.12–0.5
	Ciprofloxacin	0.03	0.03	0.008–0.03
	Cefpodoxime	0.06	0.06	0.03–0.06
	Amoxicillin-clavulanate	0.5	4	0.25–8
P. vulgaris (15)	BAY 12-8039	0.25	0.25	0.06–0.5
	Trovafloxacin	0.25	0.5	0.06–1
	Ciprofloxacin	0.03	0.03	0.008–0.03
	Cefpodoxime	0.12	0.5	0.03–0.5
	Amoxicillin-clavulanate	2	8	0.5–8
M. morganii (15)	BAY 12-8039	0.12	0.25	0.03–0.25
	Trovafloxacin	0.25	0.5	0.06–1
	Ciprofloxacin	0.008	0.015	0.004–0.015
	Cefpodoxime	0.12	4	0.015–16
	Amoxicillin-clavulanate	64	64	16–128
Serratia spp. (20) [S. marcescens (15);	BAY 12-8039	0.5	2	0.03–16
S. liquefaciens (4)]	Trovafloxacin	0.5	4	0.06–64
	Ciprofloxacin	0.12	4	0.015–16
	Cefpodoxime	4	64	1->128
	Amoxicillin-clavulanate	64	128	8->128
Acinetobacter spp. (15) [A. baumannii (11);	BAY 12-8039	0.06	2	0.008–16
A. haemolyticus (3)]	Trovafloxacin	0.03	1	0.004–16
	Ciprofloxacin	0.25	8	0.015–128
	Cefpodoxime	16	>128	1->128
	Amoxicillin-clavulanate	8	64	2->128
P. aeruginosa (15)	BAY 12-8039	2	8	0.12–64
	Trovafloxacin	0.5	8	0.03–128
	Ciprofloxacin	0.25	8	0.015–32
	Cefpodoxime	>128	>128	128->128
	Amoxicillin-clavulanate	128	>128	32->128
S. maltophilia (13)	BAY 12-8039	0.5	2	0.06–2
	Trovafloxacin	0.5	2	0.12–8
	Ciprofloxacin	2	8	0.25–16
	Cefpodoxime	>128	>128	64->128
	Amoxicillin-clavulanate	128	>128	64->128
Enterobacter spp. (5)	BAY 12-8039			0.12
	Trovafloxacin			0.06–0.12
	Ciprofloxacin			0.015–0.03
	Cefpodoxime			1->128
	Amoxicillin-clavulanate			4–128
Citrobacter spp. (5) [C. diversus (3);	BAY 12-8039			0.03–0.25
C. freundii (2)]	Trovafloxacin			0.03–0.25
	Ciprofloxacin			0.008–0.06
	Cefpodoxime			0.25–2
	Amoxicillin-clavulanate			1–32
Salmonella spp. (5)	BAY 12-8039			0.06–1
	Trovafloxacin			0.06–1
	Ciprofloxacin			0.03–0.25
	Cefpodoxime			0.5–2
	Amoxicillin-clavulanate			0.5–16
Shigella spp. (5)	BAY 12-8039			0.03–0.06
	Trovafloxacin			0.015–0.06
	Ciprofloxacin			0.015
	Cefpodoxime			0.25–0.5
	Amoxicillin-clavulanate			1–8
Providencia spp. (15) [P. stuartii (11);	BAY 12-8039	0.25	0.5	0.06–1
P. retgeri (2); P. alcalifaciens (2)]	Trovafloxacin	0.12	0.25	0.06–1

TABLE 1—Continued

Organism (no.)	Antibiotic	MIC (µg/ml)*		
		50%	90%	Range
	Ciprofloxacin	0.03	0.25	0.015–0.25
	Cefpodoxime	0.03	1	0.05–16
	Amoxicillin-clavulanate	128	128	2–128
MSSA (54)	BAY 12-8039	0.06	0.12	0.03–0.12
	Trovafloxacin	0.03	0.06	0.015–0.12
	Ciprofloxacin	0.5	1	0.12–2
	Cefpodoxime	2	4	0.5–4
	Amoxicillin-clavulanate	0.25	0.5	0.12–1
MRSA (20)	BAY 12-8039	2	2	2
	Trovafloxacin	2	2	2
	Ciprofloxacin	128	128	32–128
	Cefpodoxime	>128	>128	128->128
	Amoxicillin-clavulanate	16	16	16–32
S. epidermidis (29)	BAY 12-8039	0.06	2	0.03–2
	Trovafloxacin	0.03	4	0.15–4
	Ciprofloxacin	0.25	8	0.12–32
	Cefpodoxime	1	16	0.5->128
	Amoxicillin-clavulanate	0.12	2	0.12–64
S. saprophyticus (30)	BAY 12-8039	0.12	0.25	0.12–0.25
	Trovafloxacin	0.06	0.12	0.06–0.12
	Ciprofloxacin	0.5	0.5	0.25–0.5
	Cefpodoxime	4	8	2–8
	Amoxicillin-clavulanate	0.25	0.5	0.12–0.5
S. pneumoniae (32)	BAY 12-8039	0.12	0.25	0.06–8
	Trovafloxacin	0.12	0.25	0.06–8
	Ciprofloxacin	1	16	0.5–128
	Cefpodoxime	0.5	4	0.03–8
	Amoxicillin-clavulanate	0.12	1	0.015–1
S. milleri (30)	BAY 12-8039	0.12	0.25	0.06–0.25
	Trovafloxacin	0.12	0.25	0.06–0.25
	Ciprofloxacin	0.5	1	0.5–8
	Cefpodoxime	0.25	0.5	0.03–32
	Amoxicillin-clavulanate	0.12	0.12	0.03–0.25
Group A streptococci (20)	BAY 12-8039	0.25	0.25	0.06–0.25
	Trovafloxacin	0.12	0.25	0.06–0.25
	Ciprofloxacin	0.5	1	0.25–1
	Cefpodoxime	0.015	0.015	0.015
	Amoxicillin-clavulanate	0.015	0.015	0.015
Group B streptococci (20)	BAY 12-8039	0.25	0.25	0.06–0.5
	Trovafloxacin	0.25	0.25	0.12–0.5
	Ciprofloxacin	1	1	0.5–2
	Cefpodoxime	0.06	0.06	0.03–0.06
	Amoxicillin-clavulanate	0.06	0.06	0.06
E. faecalis (30)	BAY 12-8039	0.25	0.5	0.12–4
	Trovafloxacin	0.25	0.5	0.12–8
	Ciprofloxacin	2	2	1–32
	Cefpodoxime	8	>128	1->128
	Amoxicillin-clavulanate	0.5	0.5	0.12–16
E. faecium (20)	BAY 12-8039	2	2	0.25–4
	Trovafloxacin	0.5	2	0.25–8
	Ciprofloxacin	2	4	.1–8
	Cefpodoxime	>128	>128	0.5->128
	Amoxicillin-clavulanate	4	16	0.12–16
H. influenzae (36)	BAY 12-8039	0.03	0.03	0.015–0.06
	Trovafloxacin	0.008	0.015	0.004–0.03
	Ciprofloxacin	0.015	0.015	0.008–0.015
	Cefpodoxime	0.06	0.12	0.03–0.5
	Amoxicillin-clavulanate	0.5	2	0.25–4
M. catarrhalis (35)	BAY 12-8039	0.06	0.12	0.06–0.12
	Trovafloxacin	0.03	0.03	0.008–0.06
	Ciprofloxacin	0.06	0.06	0.03–0.06
	Cefpodoxime	0.5	1	0.12–16
	Amoxicillin-clavulanate	0.12	0.25	0.015–1
N. gonorrhoeae (34)	BAY 12-8039	0.008	0.015	0.004–0.12
	Trovafloxacin	0.004	0.008	0.002–0.03
	Ciprofloxacin	0.004	0.004	0.001–0.12

TABLE 1—*Continued*

Organism (no.)	Antibiotic	MIC (μg/ml)[a]		
		50%	90%	Range
	Cefpodoxime	0.008	0.015	0.002–0.03
	Amoxicillin-clavulanate	0.25	1	0.06–1
N. meningitidis (10)	BAY 12-8039	0.008	0.015	0.004–0.015
	Trovafloxacin	0.004	0.008	0.004–0.008
	Ciprofloxacin	0.008	0.008	0.004–0.008
	Cefpodoxime	0.004	0.004	0.004–0.008
	Amoxicillin-clavulanate	0.06	0.12	0.03–0.12
Peptostreptococcus spp. (20)	BAY 12-8039	0.12	1	0.06–1
	Trovafloxacin	0.5	1	0.06–2
	Ciprofloxacin	1	2	0.12–2
	Cefpodoxime	1	4	0.25–64
	Amoxicillin-clavulanate	0.06	0.25	0.06–16
B. fragilis (25)	BAY 12-8039	0.25	0.25	0.12–1
	Trovafloxacin	1	1	0.5–1
	Ciprofloxacin	2	4	1–4
	Cefpodoxime	64	>128	2–>128
	Amoxicillin-clavulanate	0.5	2	0.5–4
Prevotella spp. (3)	BAY 12-8039			0.12–0.25
	Trovafloxacin			0.25–1
	Ciprofloxacin			2
	Cefpodoxime			2–>128
	Amoxicillin-clavulanate			2
Clostridium perfringens (10)	BAY 12-8039	0.5	0.5	0.25–1
	Trovafloxacin	0.5	1	0.5–1
	Ciprofloxacin	0.5	0.5	0.25–0.5
	Cefpodoxime	16	32	1–32
	Amoxicillin-clavulanate	0.25	0.25	0.06–0.25
C. difficile (10)	BAY 12-8039	1	2	1–2
	Trovafloxacin	2	2	2
	Ciprofloxacin	16	16	16
	Cefpodoxime	>128	>128	128–>128
	Amoxicillin-clavulanate	0.5	1	0.25–2

[a] 50% and 90%, MIC_{50} and MIC_{90}, respectively.

RESULTS

The activity of BAY 12-8039 against members of the family *Enterobacteriaceae* (MIC at which 90% of strains tested were inhibited [MIC_{90}] ≤ 1 μg/ml, and for *Serratia* spp. MIC_{90} = 2 μg/ml) was similar to that observed for trovafloxacin (Table 1). Both these agents were generally one-half as active as ciprofloxacin, except against *Proteus mirabilis, Proteus vulgaris, Morganella morganii, Enterobacter* spp., and *Citrobacter* spp., where ciprofloxacin was 8 to 16 times more active. In general, the quinolones were more active than either of the β-lactam agents against members of the *Enterobacteriaceae*. BAY 12-8039 was equally active against β-lactamase-producing and -nonproducing strains of *E. coli*.

BAY 12-8039 was shown to be more active against *Acinetobacter* spp. (MIC_{90}, 2 μg/ml) than ciprofloxacin (MIC_{90}, 8 μg/ml). Against *P. aeruginosa* and *Stenotrophomonas maltophilia* the quinolones, including BAY 12-8039, were more active (MIC_{90}s ≤ 8 μg/ml) than the β-lactam agents (MIC_{90}s > 128 μg/ml). Both BAY 12-8039 and trovafloxacin were more active

against *S. maltophilia* (MIC_{90}s, 2 μg/ml) than ciprofloxacin (MIC_{90}s, 8 μg/ml).

BAY 12-8039 exhibited activity against *Staphylococcus saprophyticus* (MIC_{90}, 0.25 μg/ml) and *Staphylococcus epidermidis* (MIC_{90}, 2 μg/ml), the MIC_{90}s of ciprofloxacin being 0.5 and 8 μg/ml, respectively. The activity of BAY 12-8039 against MSSA (MIC_{90}, 0.12 μg/ml) was similar to that of trovafloxacin (MIC_{90}, 0.06 μg/ml) but greater than that of ciprofloxacin (MIC_{90}, 1 μg/ml). BAY 12-8039 was less active against methicillin-resistant *S. aureus* (MRSA) (MIC_{90}, 2 μg/ml) than against methicillin-susceptible strains (MIC_{90}, 0.12 μg/ml). However, it was more active than ciprofloxacin (MIC_{90}, 128 μg/ml), cefpodoxime (MIC_{90}s, >128 μg/ml), and amoxicillin-clavulanate (16 μg/ml) against the MRSA.

TABLE 2. In vitro activity of BAY 12-8039 in comparison with rifampin against *M. tuberculosis*

Strain	Resistance pattern to commonly used antimycobacterial agents	MIC (μg/ml) of:	
		BAY 12-8039	Rifampin
1	Fully sensitive	0.5	0.25
2	Isoniazid and streptomycin resistant	0.25	0.25
3	Isoniazid and rifampin resistant	0.12	ND[a]
4	Streptomycin resistant	0.25	0.5

[a] ND, not determined.

TABLE 3. MIC and MLC of BAY 12-8039 and comparator
agents for *C. trachomatis* and *C. pneumoniae*[a]

Strain	BAY 12-8039		Ciprofloxacin		Erythromycin	
	MIC	MLC	MIC	MLC	MIC	MLC
C. trachomatis 6/96	0.06	0.12	2.0	2.0	0.25	2.0
C. trachomatis 7/96	0.12	0.12	2.0	2.0	0.5	4.0
C. trachomatis 8/96	0.06	0.12	1.0	2.0	0.5	4.0
C. pneumoniae TW183	0.06	0.06	2.0	2.0	0.25	0.5

[a] Values are given in micrograms per milliliter.

BAY 12-8039 exhibited activity against *Streptococcus milleri* and group A and group B streptococci (MIC90s, 0.25 μg/ml), and this was comparable to that of trovafloxacin. The activity of BAY 12-8039 against *S. pneumoniae* (MIC90, 0.25 μg/ml) was also similar to that of trovafloxacin but was considerably greater than that of ciprofloxacin (MIC90, 16 μg/ml). A strain inhibited by 16 μg of ciprofloxacin per ml was inhibited by 0.12 and 0.25 μg of BAY 12-8039 and trovafloxacin per ml, respectively. BAY 12-8039 was also shown to be active against *E. faecalis* (MIC90, 0.5 μg/ml) and *Enterococcus faecium* (MIC90, 2 μg/ml).

BAY 12-8039, in common with the other quinolones, was highly active against *Neisseria gonorrhoeae* and *Neisseria meningitidis* (MIC90s, 0.015 μg/ml), *H. influenzae* (MIC90, 0.03 μg/ml), and *M. catarrhalis* (MIC90, 0.12 μg/ml).

BAY 12-8039 was active against all the strains of anaerobic bacteria studied (MIC90s ≤ 2 μg/ml). BAY 12-8039 was three or fourfold more active against *Bacteroides fragilis*, *Prevotella* spp., and *Clostridium difficile* than ciprofloxacin.

BAY 12-8039 exhibited an activity comparable to that of rifampin for all strains of *M. tuberculosis* (Table 2).

Against both *C. trachomatis* and *C. pneumoniae* (Table 3) BAY 12-8039 was shown to be more active (MICs of 0.06 to 0.12 μg/ml) than either ciprofloxacin (MICs of 1 to 2 μg/ml) or erythromycin (MICs of 0.25 to 0.5 μg/ml). BAY 12-8039 exhibited a high lethal effect against both *C. trachomatis* and *C. pneumoniae*, with the MLCs being equal to, or within one dilutional step of, the MICs.

An increase in inoculum size from 10⁴ to 10⁶ did not affect the MICs for the *E. coli* strains studied (data not shown). For *K. pneumoniae*, however, one strain was affected, and in this case the MIC increased fourfold. The majority of *P. mirabilis* strains tested at an increased inoculum showed a twofold in-

crease in MIC. For *S. marcescens*, two of five strains showed a threefold increase in MIC.

The presence of human serum had no marked effect on the MICs or MBCs determined for BAY 12-8039 at either 20 or 70% (Table 4), with the exception of one strain of group A streptococci for which the MBC was 0.25 μg/ml in the absence of serum and 1 μg/ml in the presence of 70% serum. The protein binding of BAY 12-8039 was determined at 1 and 5 μg/ml as 30 and 26.4%, respectively.

DISCUSSION

The results presented here generally agree with preliminary information on BAY 12-8039 which indicates improved in vitro activity against gram-positive bacteria (3). In this study, BAY 12-8039 was found to be more active than ciprofloxacin against *S. pneumoniae*, MSSA, and MRSA. In addition, the activity of BAY 12-8039 equalled that of trovafloxacin, which has previously been shown to possess improved activity against gram-positive bacteria (2, 4). It should be noted that BAY 12-8039 was less active against MRSA than against MSSA. The strains of MRSA used in this study were recent clinical isolates, and it is therefore likely that some were ciprofloxacin-resistant epidemic MRSA (6). In the clinical situation resistance to ciprofloxacin by MRSA appears to be rapidly acquired (7, 9), and it is possible that the mechanism(s) of resistance to ciprofloxacin also applies to BAY 12-8039.

In common with other fluoroquinolones, BAY 12-8039 exhibited activity against the *Enterobacteriaceae*. Against *Acinetobacter* spp. BAY 12-8039 was shown to be more active than ciprofloxacin. In addition, BAY 12-8039 was generally found to have improved activity compared to that of ciprofloxacin against anaerobic bacteria.

BAY 12-8039 was shown to be active against common respiratory pathogens, such as *M. catarrhalis* and *H. influenzae*. Against *M. tuberculosis* BAY 12-8039 was found to be as active as rifampin. This activity is similar to that of ciprofloxacin and improved compared to that of trovafloxacin (2). BAY 12-8039 was shown to be slightly more active against *Mycobacterium avium-Mycobacterium intracellulare* compared to rifampin. Against strains of *Chlamydia* spp. BAY 12-8039 was found to be more active than either erythromycin or ciprofloxacin.

The protein binding of BAY 12-8039 was similar to that of many other fluoroquinolones (<50%) but less than that of trovafloxacin, which we found to be approximately 85% em-

TABLE 4. Effect of human serum on the in vitro activity of BAY 12-8039

Organism type[a]	Agar MIC (μg/ml)	Broth MIC (μg/ml)	MBC (μg/ml)	20% Human serum		70% Human serum	
				MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)
Group A streptococci	0.25	0.25	0.25	0.25	0.25	0.5	1
	0.25	0.12	0.12	0.25	0.25	0.25	0.25
S. pneumoniae	0.12	0.25	0.25	0.12	0.12	0.12	0.5
	0.25	0.25	0.25	0.25	0.25	0.25	0.5
M. catarrhalis	0.12	0.06	0.12	0.03	0.12	ND[b]	0.25
	0.12	0.06	0.12	ND	0.12	0.03	0.25
S. aureus	0.03	0.03	0.06	0.06	0.12	0.06	0.12
	0.06	0.03	0.06	0.06	0.12	0.06	0.12
E. coli	0.06	0.03	0.06	0.03	0.03	0.03	0.03
	0.03	0.03	0.03	0.015	0.03	0.03	0.03
K. pneumoniae	0.06	0.06	0.12	0.12	0.12	0.12	0.12
	0.12	0.06	0.06	0.03	0.06	0.03	0.06

[a] Two strains of each organism were studied.
[b] ND, not determined.

ploying similar methodologies (2). The presence of serum had, as expected, little or no effect upon the in vitro antimicrobial activity of the new compound.

BAY 12-8039 has a broad spectrum of activity which includes gram-negative and gram-positive bacteria, *Chlamydia* spp., *M. tuberculosis*, and anaerobes and therefore has considerable clinical potential in a wide range of infections.

ACKNOWLEDGMENT

REFERENCES

1. **British Society for Antimicrobial Chemotherapy Working Party.** 1991. A guide to sensitivity testing. British Society for Antimicrobial Chemotherapy, London, United Kingdom.
2. **Child, J., J. M. Andrews, F. Boswell, N. P. Brenwald, and R. Wise.** 1995. The in-vitro activity of CP99219, a new naphthyridine antimicrobial agent: a comparison with fluoroquinolone agents. J. Antimicrob. Chemother. 35:869–876.
3. **Dalhoff, A., U. Peterson, and R. Endermann.** In vitro activity of BAY 12-8039, a new 8-methoxyquinolone. Chemotherapy (Basel), in press.
4. **Eliopoulos, G. M., K. Klimm, C. T. Eliopoulos, M. J. Ferraro, and R. C. Moellering.** 1993. In vitro activity of CF 99,219, a new fluoroquinolone, against clinical isolates of gram-positive bacteria. Antimicrob. Agents Chemother. 37:366–370.
5. **Endermann, R., and K. D. Bremm.** 1992. BAY y3118, a novel 4-quinone: activity against anaerobes, abstr. 645. *In* Program and abstracts of the 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
6. **Hospital Infection Society Working Party.** 1995. Guidelines on the control of methicillin-resistant Staphylococcus aureus in the community. J. Hosp. Infect. 31:1–12.
7. **Isaacs, R. D., P. J. Kunke, R. L. Cohen, and J. W. Smith.** 1988. Ciprofloxacin resistance in epidemic methicillin resistant *Staphylococcus aureus*. Lancet ii:843.
7a. **Pearson, R. D., R. T. Steigbel, H. T. Davis, and S. W. Chapman.** 1980. Method for reliable determination of minimum lethal antibiotic concentrations. Antimicrob. Agents Chemother. 18:690–708.
8. **Perrone, C., A. Gikas, C. Tuffot-Pernot, J. Grosset, J.-J. Pocidalo, and J.-L. Vilde.** 1990. Activities of clarithromycin, sulfisoxazole, and rifabutin against *Mycobacterium avium* complex multiplication within human macrophages. Antimicrob. Agents Chemother. 34:1508–1511.
9. **Rayiglione, M. C., J. F. Boyle, P. Mariux, A. Pablos-Mendez, H. Cortes, and A. Merlo.** 1990. Ciprofloxacin-resistant methicillin-resistant *Staphylococcus aureus* in an acute-care hospital. Antimicrob. Agents Chemother. 34:2050–2054.
10. **Wakabayashi, E., and S. Mitsuhashi.** 1994. In vitro activity of AM-1155, a novel 6-fluoro-3-methoxy quinolone. Antimicrob. Agents Chemother. 38: 594–601.
11. **Webberley, J. M., R. S. Matthews, J. M. Andrews, and R. Wise.** 1987. Commercially available fluorescein-conjugated monoclonal antibody for determining the in vitro activity of antimicrobial agents against *Chlamydia trachomatis*. Eur. J. Clin. Microbiol. 6:587–589.

Pseudomonas aeruginosa keratitis in leukopenic rabbits

Jeffery A.Hobden[1], Lee S.Engel[2], Michelle C.Callegan[2], James M.Hill[1,2], Bryan M.Gebhardt[1] and Richard J.O'Callaghan[2]

[1]Lions Eye Research Laboratories, LSU Eye Center and [2]Department of Microbiology, Immunology and Parasitology, Louisiana State University Medical Center School of Medicine, New Orleans, LA 70112, USA

ABSTRACT
To study the role of the host inflammatory response in *Pseudomonas aeruginosa* keratitis, rabbits were made leukopenic with intravenous injections of cyclophosphamide and dexamethasone. Twenty-four hr later, keratitis was initiated in all rabbits with an intrastromal injection of 1,000 log phase *P. aeruginosa* strain 27853. Slit lamp examination of eyes showed that leukopenic rabbits had significantly less (P<0.0001) ocular pathology at 16, 22, and 27 hr postinfection. The number of viable bacteria recovered from corneas of leukopenic rabbits was the same as the number recovered from nonleukopenic rabbits (P=0.95). These results suggest that the host inflammatory response significantly contributes to the overall ocular pathology associated with *P. aeruginosa* keratitis, but does not influence the survival of the infecting organism in the cornea at the height of the infection.

INTRODUCTION
Pseudomonas aeruginosa causes the most severe form of bacterial keratitis (1,2). Infections of the cornea with this organism are characterized by a rapid liquefactive necrosis of the stroma (3), which can lead to corneal perforation within 24 hr (2).

The pathogenesis of *P. aeruginosa* keratitis appears to involve both bacterial (4-6) and host (7-9) constituents. Bacterial factors reported to be important for ocular virulence are elastase, alkaline protease, and exotoxin A (6,10). Host-derived products which could damage the cornea are thought to be associated with polymorphonuclear

leukocytes (PMN) chemotactically attracted to the site of infection (11-15).

The exact role bacterial and host factors play in the destruction of the cornea is unknown. To determine the role of the host inflammatory response in *P. aeruginosa* keratitis, rabbits in this study were made leukopenic prior to being intrastromally infected with viable *P. aeruginosa*. The development of ocular pathology in these rabbits was recorded by slit lamp examination (SLE) at 16, 22, and 27 hr postinfection (PI). The effects of leukopenia on the number of PMN infiltrating the infected cornea, as well as on the number of viable bacteria present in corneal tissue, were determined 27 hr PI.

MATERIALS AND METHODS
Induction of leukopenia
All animals were treated in accordance with the ARVO Resolution on the Use of Animals in Research. New Zealand white rabbits (3.0±0.1 kg) were made leukopenic with injections of cyclophosphamide (75 mg/kg) and dexamethasone (4 mg/kg) as described by Stroop and Schaefer (16). Rabbits were anesthetized with an intramuscular injection of ketamine hydrochloride (20 mg/kg; Ketaset®, 100 mg/ml, Aveco Co., Inc., Fort Dodge, IA) and xylazine hydrochloride (10 mg/kg; Rompun®, 100 mg/ml, Miles Laboratories,

APPENDIX C

Shawnee, KS). A 20 mg/ml filter-
sterilized solution of cyclophosphamide
monohydrate (Sigma Chemicals, St. Louis,
MO) prepared in phosphate buffered saline
(PBS, pH 7.4) was then injected into the
marginal ear vein. Control rabbits were
injected with PBS (3 ml/kg). Twenty-four
hours later, dexamethasone sodium
phosphate (4 mg/ml; The Butler Co.,
Columbus, MO) or PBS (1 ml/kg) was
intravenously administered. White blood
cell (WBC) counts were determined in each
rabbit from blood drawn from the marginal
ear vein using a Unipette kit (Becton-
Dickinson, Rutherford, NJ) for manual WBC
determinations prior to each injection,
at the initiation of infection, and at
the time of sacrifice. To differentiate
the WBC types at the time of sacrifice
(27 hr PI), blood smears were prepared
and stained with Wright's Stain
(Leukostat®, Fisher Diagnostics,
Orangeburg, NY).

Experimental *Pseudomonas aeruginosa*
keratitis

P. aeruginosa keratitis was initiated at
24 hr after the injection of
dexamethasone in leukopenic and
nonleukopenic rabbits (3 rabbits, 6 eyes
per group). The procedure for initiating
keratitis has been described (17).
Briefly, rabbits were anesthetized with
ketamine and xylazine as described above
and corneas were anesthetized with 0.5%
proparacaine hydrochloride (Ophthaine®,
E.R. Squibb & Sons, Inc., New Brunswick,
NJ). An aliquot of 10 μl of tryptic soy
broth (Difco Laboratories, Detroit, MI)
containing approximately 1,000
logarithmically grown *P. aeruginosa* ATCC
27853 was then injected into the stroma.
P. aeruginosa 27853 establishes
reproducible keratitis in rabbits (18-22)
and guinea pigs (23,24).

Evaluation of ocular inflammation

Slit lamp examination and estimations of

numbers of PMN infiltrating into corneal
tissue was used to determine the effect
of leukopenia on the inflammatory
response resulting from the pseudomonal
corneal infection.

To assess inflammatory changes in the
conjunctiva, anterior chamber, and the
cornea, eyes were examined with a Topcon
SL-5D slit lamp biomicroscope (Kogaku
Kikai K.K., Tokyo, Japan) at 16, 22, and
27 hr PI. All examinations were conducted
independently in a masked fashion by
three observers. The scoring system used
has been previously described (22,25).
Briefly, scores of 0.00 (absent) to +4.00
(severe) in 0.25 increments were assigned
to seven parameters: conjunctival
injection, conjunctival chemosis, iritis
(cell and flare), fibrin in anterior
chamber, hypopyon, stromal infiltrate,
and stromal edema. Scores from each of
the parameters were summed to provide a
single value that represented the degree
of change observed.

The numbers of PMN in corneal tissue at
27 hr PI was determined by quantitating
myeloperoxidase (MPO) activity in an
assay similar to that described by
Williams et al. (26). Assays were
conducted in 96-well microtiter plates
(Costar; Cambridge, MA) and samples were
run in triplicate. Rabbits were
sacrificed with an overdose of
pentobarbital sodium (The Butler Co.) and
corneas were aseptically removed as
previously described (17). Corneas were
homogenized as described below for
quantitation of viable *P. aeruginosa* per
cornea. Aliquots of 0.1 ml of homogenate
were removed for bacterial enumeration
before hexadecyltrimethylammonium bromide
(CTAB, Sigma) was added to a final
concentration of 0.5%. The final volume
of the homogenate was 3.0 ml. The mixture
was further homogenized on ice for 30
sec. Tissue debris was removed from the

462

homogenate by centrifugation at 40,000 x g for 15 min at 4°C. A 6.9 µl aliquot of the resulting supernatant was then mixed with 200 µl of potassium phosphate buffer (50 mM, pH 6.0) containing o-dianisidine dihydrochloride (16.7 mg/100 ml, Sigma) and hydrogen peroxide (0.0005%). Optical density at 450 nm was measured every 2 min with a Dynatech MR500 microtiter plate reader (Dynatech Laboratories, Chantilly, VA) for 10-15 min at room temperature. Calculations of MPO activity were performed as described by Williams et al. (26). One unit of MPO activity is equivalent to approximately 5 logs of PMN. The lowest detectable MPO activity was 0.01 units which is equivalent to approximately 3 logs of PMN. For corneas with less than 0.01 units, a value of 0 PMN per cornea was used to calculate the average number of PMN per group. PMN determinations are expressed as the log base 10 number of PMN per cornea.

Quantitation of viable P. aeruginosa per cornea

Corneas surgically removed at the corneoscleral limbus were minced, placed into a sterile tube containing 3.0 ml sterile PBS (pH 7.4), and homogenized on ice with an Ultra-Turrax® Tissuemizer (Tekmar Co., Cincinnati, OH). Homogenates were serially diluted (1:10) to a dilution factor of 10^{-6}. Aliquots (0.1 ml) of each dilution (including the undiluted sample) were plated on tryptic soy agar plates (Difco Laboratories, Detroit, MI) and incubated for 24-48 hr at 37°C.

Statistical analysis of data

Statistical analysis was carried out using the Statistical Analysis System (SAS) software program (27) for personal computers. For colony forming units and log number of PMN, an analysis of variance was performed and, where a significant analysis of variance was

found, t tests between the least square means from each treatment group were performed. For SLE scores, nonparametric one-way analysis of variance (Kruskal-Wallis test) was used. For comparison among groups in this analysis, Wilcoxon scores were used. A probability value of less than 0.05 was considered significant.

RESULTS

The decrease in circulating WBC after treatment with cyclophosphamide and dexamethasone is shown in Figure 1. The number of circulating WBC in drug-treated rabbits compared to control rabbits decreased 40% by day 2 (24 hours after dexamethasone injection) and even further to 80% by day 3. Peripheral blood smears showed a corresponding 50% decrease in circulating PMN in the leukopenic rabbits, compared to the nonleukopenic rabbits, at the time of sacrifice (day 3).

The infected, leukopenic rabbits demonstrated significantly less ocular pathology at 16, 22, and 27 hr (P<0.0001), compared to the nonleukopenic animals (Table 1). As in the peripheral circulation, the corneas from infected leukopenic rabbits had significantly fewer PMN than the corneas from nonleukopenic rabbits (P<0.02) (Table 2). The numbers of viable bacteria, however, were not significantly different between the two groups (P=0.30) (Table 2).

DISCUSSION

Infection of mice (28), guinea pigs (29), and rabbits (30) has shown that the host inflammatory response to P. aeruginosa keratitis consists almost entirely of infiltrating PMN. These cells migrate through the corneal stroma from limbal blood vessels (31) to the site of infection in response to chemotactic
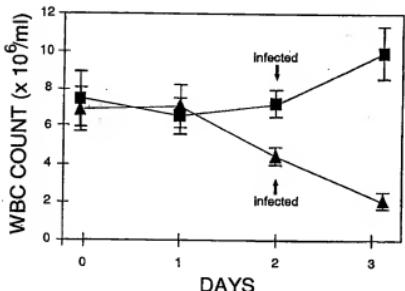
Figure 1: Induction of leukopenia in rabbits. Rabbits were made leukopenic with intravenous injections of cyclophosphamide (75 mg/kg) and dexamethasone (4 mg/kg). A sterile 20 mg/ml solution of cyclophosphamide monohydrate prepared in phosphate buffered saline (pH 7.4) was injected into the marginal ear vein on day 0. Twenty-four hr later (day 1), an injection of dexamethasone sodium phosphate (4 mg/ml) was administered intravenously. Control rabbits were injected each day with the appropriate volume of saline. The total white blood cell counts were determined in blood samples drawn from the marginal ear vein prior to the injection of cyclophosphamide (triangle) or saline (square) on day 0, and for the next 3 consecutive days. Rabbits were leukopenic 24 hr after the injection of dexamethasone (day 2) at which time bacterial keratitis was initiated. Values are means ± SEM of 3 rabbits.

stimuli originating from both the host (32,33) and the micro-organism (34). The oxidative burst in PMN and the subsequent release of free radicals and proteases (serine protease, elastase, collagenase, and gelatinase) cause extensive damage to the corneal stroma (11-15).

In 1979, Chusid and Davis (35) rendered guinea pigs neutropenic with whole body X-irradiation before intrastromally injecting an overnight culture of a strain of *P. aeruginosa* obtained from a human corneal ulcer. They reported that corneas of neutropenic guinea pigs contained one-third as many PMN and one hundred times more bacteria than corneas of non-neutropenic guinea pigs. In our study, we noted significantly fewer PMN in the corneas of leukopenic animals compared to the corneas of nonleukopenic animals. However, despite containing 2.5 logs fewer PMN, corneas of leukopenic rabbits had the same number of bacteria as the corneas of nonleukopenic rabbits. In contrast to the guinea pig model, in our rabbit model PMN were apparently unable to contain the rapid growth of bacteria.

In our study, eyes of infected leukopenic rabbits were significantly

Table 1: Slit lamp examination scores as a measure of corneal inflammation in *P. aeruginosa*-infected leukopenic rabbits

Group[1]	Leukopenic[2]	SLE[3]		
		16 hr	22 hr	27 hr
1	Yes	3.2 ± 0.6	4.7 ± 0.8	4.8 ± 1.0
2	No	6.3 ± 0.7	9.7 ± 0.8	14.7 ± 1.8

[1] Each group consisted of 3 rabbits, 6 eyes.
[2] Leukopenia induced by injection of cyclophosphamide (75 mg/kg) followed 24 hr later by dexamethasone (4 mg/kg).
[3] Slit lamp examination scores at 16, 22, and 27 hours after inoculation of *P. aeruginosa*; group 1 scores are significantly lower than group 2 scores ($P<0.0001$) at all three time points.

Table 2: *P. aeruginosa* keratitis in leukopenic rabbits

Group[1]	Leukopenia[2] Present	WBC[3]	Inflammatory cells (Log$_{10}$ PMN)[4]	Viable bacteria (Log$_{10}$ CFU)[5]
1	Yes	2.1 ± 0.3	2.6 ± 0.9	6.8 ± 0.1
2	No	10.0 ± 1.3	5.1 ± 0.2	7.0 ± 0.1

[1] Each group consisted of 3 rabbits, 6 eyes.
[2] Leukopenia induced by injection of cyclophosphamide (75 mg/kg) followed 24 hours later by dexamethasone (4 mg/kg).
[3] WBC = number of white blood cells (x 10^6/ml) in peripheral blood on the day rabbits were sacrificed (3 days after injection of cyclophosphamide); group 1 is significantly different from group 2 (P<0.0005).
[4] PMN = Log$_{10}$ polymorphonuclear leukocytes per cornea; group 1 is significantly different from group 2 (P<0.02).
[5] CFU = Log$_{10}$ colony forming units per cornea; group 1 is not significantly different from group 2 (P=0.30).

less inflamed, as judged by SLE, than eyes of infected nonleukopenic rabbits. These results are compatible with observations made by other investigators studying the relationship between leukopenia and ocular inflammation. Harrison et al. (36) and Trinkaus-Randall et al. (14) induced leukopenia in rabbits with nitrogen mustard and noticed a dampened inflammatory response in the eye when corneas were intrastromally injected with pneumolysin and lipopolysaccharide, respectively. Nanda et al. (37) reported a case of *P. aeruginosa* corneoscleritis in an HIV-infected neutropenic patient presenting with minimal symptoms of infection. Hazlett et al (38) induced leukopenia in outbred Swiss-Webster mice with cyclophosphamide and noticed less severe corneal histopathology in *P. aeruginosa*-infected leukopenic mice; however, the majority of these mice died of septicemia.

In conclusion, the host cellular inflammatory response (predominantly an influx of PMN) appears to contribute significantly to the ocular pathology observed in *P. aeruginosa* keratitis, as evidenced by the significantly lower PMN numbers and SLE scores of leukopenic rabbits.

CORRESPONDING AUTHOR
James M. Hill, Ph.D., LSU Eye Center, 2020 Gravier Street, Suite B, New Orleans, LA 70112-2234, USA.

REFERENCES
1. Liesegang, T.J. (1988) Bacterial and fungal keratitis. In "The Cornea", (eds. Kaufman, H.E., Barron, B.A., McDonald, M.B. and Waltman, S.R.). Pp. 217-270. Churchill Livingstone, Inc., New York.
2. Laibson, P.R. (1990) *Pseudomonas aeruginosa*. In "Current Ocular Therapy", (eds. Fraunfelder, F.T. and Roy, F.H.). Pp. 35-37. W.B. Saunders Company, Philadelphia.

3. Holland, S.P., Pulido, J.S., Shires, T.K. and Costerton, J.W. (1993) Pseudomonas aeruginosa ocular infections. In "Pseudomonas Aeruginosa: the Opportunist", (ed. Fick, Jr., R.B.). Pp. 159-176. CRC Press, Inc., Boca Raton, Florida.

4. Ohman, D.E., Burns, R.P. and Iglewski, B.H. (1980) Corneal infections in mice with toxin A and elastase mutant of Pseudomonas aeruginosa. J. Infect. Dis. 142, 547-555.

5. Howe, T.R. and Iglewski, B.H. (1984) Isolation and characterization of alkaline protease-deficient mutants of Pseudomonas aeruginosa in vitro and in a mouse eye model. Infect. Immun. 43, 1058-1063.

6. Nicas, T.I. and Iglewski, B.H. (1985) The contribution of exoproducts to virulence of Pseudomonas aeruginosa. Can. J. Microbiol. 31, 387-392.

7. Thiel, H.J., Steuhl, K.P. and Doring, G. (1987) Therapy of Pseudomonas aeruginosa. Antibiot. Chemother. 39, 92-101.

8. Kessler, E., Mondino, B.J. and Brown, S.I. (1977) The corneal response to Pseudomonas aeruginosa: Histopathological and enzymatic characterization. Invest. Ophthalmol. Vis. Sci. 16, 116-125.

9. Twining, S.S., Lohr, K.M. and Moulder, J.E. (1986) The immune system in experimental Pseudomonas keratitis. Invest. Ophthalmol. Vis. Sci. 21, 507-515.

10. Parmely, M.J. (1993) Pseudomonas metalloproteases and the host-microbe relationship. In "Pseudomonas aeruginosa: The Opportunist", (ed. Fick, R.B., Jr.). Pp. 79-94. CRC Press, Boca Raton, Florida.

11. Steuhl, K.P., Doring, G., Henni, A., Thiel, H.J. and Botzenhart, K. (1987) Relevance of host-derived and bacterial factors in Pseudomonas aeruginosa corneal infections. Invest. Ophthalmol. Vis. Sci. 28, 1559-1568.

12. Foster, C.S. (1988) Basic ocular immunology. In "The Cornea", (eds. Kaufman, H.E., Barron, B.A., McDonald, M.B. and Waltman, S.R.). Pp. 85-122. Churchill Livingstone, Inc., New York.

13. Weiss, J. (1989) Tissue destruction by neutrophils. N. Engl. J. Med. 320, 365-375.

14. Trinkaus-Randall, V., Leibowitz, H.M., Ryan, W.J. and Kupferman, A. (1991) Quantification of stromal destruction in the inflamed cornea. Invest. Ophthalmol. Vis. Sci. 32, 603-609.

15. Carubelli, R., Nordquist, R.E. and Rowsey, J.J. (1990) Role of active oxygen species in corneal ulceration: Effect of hydrogen peroxide generated in situ. Cornea, 9, 161-169.

16. Stroop, W.G. and Schaefer, D.C. (1987) Severity of experimentally reactivated herpetic eye disease is related to the neurovirulence of the latent virus. Invest. Ophthalmol. Vis. Sci. 28, 229-237.

17. Rootman, D.S., Hobden, J.A., Jantzen, J.A., Gonzalez, J.R., O'Callaghan, R.J. and Hill, J.M. (1988) Iontophoresis of tobramycin for the treatment of experimental Pseudomonas keratitis in the rabbit. Arch. Ophthalmol. 106, 262-265.

18. Hobden, J.A., Reidy, J.J., O'Callaghan, R.J., Hill, J.M., Insler, M.S. and Rootman, D.S. (1988) Treatment of experimental Pseudomonas keratitis using collagen shields containing tobramycin. Arch. Ophthalmol. 106, 1605-1607.

19. Hobden, J.A., O'Callaghan, R.J., Hill, J.M., Reidy, J.J., Rootman, D.S. and Thompson, H.W. (1989) Tobramycin iontophoresis into corneas infected with drug-resistant Pseudomonas aeruginosa. Curr. Eye Res. 8, 1163-1169.

20. Hobden, J.A., Reidy, J.J., O'Callaghan, R.J., Insler, M.S. and Hill, J.M. (1990) Ciprofloxacin iontophoresis for aminoglycoside-resistant pseudomonal keratitis. Invest. Ophthalmol. Vis. Sci. 31, 1940-1944.

21. Reidy, J.J., Hobden, J.A., Hill, J.M., Forman, K. and O'Callaghan, R.J. (1991) The efficacy of topical ciprofloxacin and norfloxacin in the treatment of experimental Pseudomonas keratitis. Cornea, 10, 25-28.

22. Hobden, J.A., O'Callaghan, R.J., Hill, J.M., Hagenah, M., Insler, M.S. and Reidy, J.J. (1992) Ciprofloxacin and prednisolone therapy for experimental Pseudomonas keratitis. Curr. Eye Res. 11, 259-266.

23. Gritz, D.C., Lee, T.Y., Kwitko, S. and McDonnell, P.J. (1990) Topical antiinflammatory agents in an animal model of microbial keratitis. Arch. Ophthalmol. 108, 1001-1005.

24. Ohadi, C., Litwin, K.L., Moreira, H., Kwitko, S., Gauderman, W.J., Gritz, D.C., Gwon, A., Jones, R. and McDonnell, P.J. (1992) Anti-inflammatory therapy and outcome in a guinea pig model of Pseudomonas keratitis. Cornea, 11, 398-403.

25. Johnson, M.K., Hobden, J.A., Hagenah, M., O'Callaghan, R.J.,

Hill, J.M. and Chen, S. (1990) The role of pneumolysin in ocular infections with *Streptococcus pneumoniae*. Curr. Eye Res. 2, 1107-1114.

26. Williams, R.N., Paterson, C.A., Eakins, K.E. and Bhattacherjee, P. (1982/1983) Quantification of ocular inflammation: Evaluation of polymorphonuclear leukocyte infiltration by measuring myeloperoxidase activity. Curr. Eye Res. 2, 465-470.

27. SAS Institute, Inc. (1985) SAS Users Guide: Statistics, Version 5 Edition. SAS Institute, Cary, N.C.

28. Hazlett, L.D., Zucker, M. and Berk, R.S. (1992) Distribution and kinetics of the inflammatory cell response to ocular challenge with *Pseudomonas aeruginosa* in susceptible versus resistant mice. Ophthalmic Res. 24, 32-39.

29. van Horn, D.L., Davis, S.D., Hyndiuk, R.A. and Alpren, T.V.P. (1978) Pathogenesis of experimental *Pseudomonas* keratitis in the guinea pig: Bacteriologic, clinical, and microscopic observations. Invest. Ophthalmol. Vis. Sci. 17, 1076-1086.

30. van Horn, D.L., Davis, S.D., Hyndiuk, R.A. and Pederson, H.J. (1981) Experimental *Pseudomonas* keratitis in the rabbit: Bacteriologic, clinical, and microscopic observations. Invest. Ophthalmol. Vis. Sci. 20, 213-221.

31. Chusid, M.J. and Davis, S.D. (1985) Polymorphonuclear leukocyte kinetics in experimentally induced keratitis. Arch. Ophthalmol. 103, 270-274.

32. Ben-Zvi, A., Rodrigues, M.M., Gery, I. and Schiffmann, E. (1981) Induction of ocular inflammation by synthetic mediators. Arch. Ophthalmol. 99, 1436-1444.

33. Elgebaly, S.A., Miano, D.C., Kreutzer, D.L. and Fishman, J.B. (1990) Cornea derived neutrophil chemotactic factors: Intracellular synthesis and release. Curr. Eye Res. 2, 839-845.

34. Fontan, P.A., Amura, C.R., Garcia, V.E., Cerquetti, M.C. and Sordelli, D.O. (1992) Preliminary characterization of *Pseudomonas aeruginosa* peptide chemotactins for polymorphonuclear leukocytes. Infect. Immun. 60, 2465-2469.

35. Chusid, M.J. and Davis, S.D. (1979) Experimental bacterial keratitis in neutropenic guinea pigs: Polymorphonuclear leukocytes in corneal host defense. Infect. Immun. 24, 948-952.

36. Harrison, J.C., Karcioglu, Z.A. and Johnson, M.K. (1982/83) Response of leukopenic rabbits to pneumococcal toxin. Curr. Eye Res. 2, 705-710.

37. Nanda, M., Pflugfelder, S.C. and Holland, M. (1991) Fulminant pseudomonal keratitis and scleritis in human immunodeficiency virus-infected patients. Arch. Ophthalmol. 109, 503-505.

38. Hazlett, L.D., Rosen, D.D. and Berk, R.S. (1977) *Pseudomonas* eye infections in cyclophosphamide-treated mice. Invest. Ophthalmol. Vis. Sci. 16, 649-652.

TECHNICAL REPORT

Archives File No.:

Unit: R&D Microbiology

Project Name (Number):   Anti-Infective Program

Title: *In Vivo* Evaluation of Moxifloxacin (AL-15469A) in Rabbit
                                   Keratitis Model

Author(s):   David W. Stroman and Richard O'Callaghan (LSU)

Name of Study Director: Richard O'Callaghan, Ph.D.

Name of Study Monitor: David W. Stroman, Ph.D.
(if a contract study)

Name/Address of   Department of Microbiology
Testing Facility: Louisiana State University Medical School
                                   1901 Perdido St.
                                   New Orleans, LA 70112

Acknowledgments:

Key Words: Moxifloxacin
                                   Ciprofloxacin
                                   Rabbit Keratitis Model

Distribution: QAU/Archives (Original)

Approval(s):

David W. Stroman, Ph.D.   4/28/99   Barry A. Schlech, Ph.D.   4/28/99
Associate Director, Anti-Infective   Date   Vice President, Pharmaceutical   Date
Program   Sciences

Summary: Moxifloxacin was evaluated for its ability to kill *Pseudomonas
                                   aeruginosa* and *Staphylococcus aureus* in the rabbit
                                   intrastromal model of keratitis. Topically applied moxifloxacin
                                   (0.3%) was shown to be equally active as CILOXAN (0.3%
                                   ciprofloxacin) in killing *P. aeruginosa* and *S. aureus* in the
                                   cornea. These data are very positive, especially in view of the
                                   fact that *in vitro* moxifloxacin is approximately 8 times less
                                   active than ciprofloxacin against *P. aeruginosa*.

APPENDIX D

00045265 99118-003

051

1. INTRODUCTION

An *in vivo* rabbit keratitis model has been developed and utilized by Richard O'Callaghan et al. (LSU Medical School) for several years to evaluate and compare ophthalmic formulations of anti-bacterial agents and their ability to eradicate pathogenic bacteria injected intrastromally from the cornea (Hobden, J. A. et al., 1993).

2. METHODS AND MATERIALS

2.1.   Formulations of Moxifloxacin

Three different concentrations of moxifloxacin were prepared and tested. The moxifloxacin vehicle (FID 99916) contained boric acid - 0.155%; sodium chloride – 0.85%; disodium EDTA – 0.05%; benzalkonium chloride – 0.006%; pH adjusted to 7.5. The FID numbers for the three moxifloxacin formulations were FID 99905 - 0.2% moxifloxacin; FID 99906 - 0.3% moxifloxacin; and FID 99907 – 0.5% moxifloxacin.

2.2.   Details of Infection Models

The experimental design of the infection models used have been published previously (Hobden, J. A. et al., 1993).

3. RESULTS AND DISCUSSION

Three different concentrations of moxifloxacin were evaluated for their ability to kill *Pseudomonas aeruginosa* and *Staphylococcus aureus* in the rabbit intrastromal model of keratitis. In both infection models, conditions of therapy were chosen such that ciprofloxacin did not completely sterilize the cornea; therefore, moxifloxacin could be evaluated as comparable to ciprofloxacin, more active than ciprofloxacin, or less active than ciprofloxacin.

TR No.: 023:38540:0399
Protocol No.: N/A

3 of 3

Alcon
LABORATORIES

Revision No.: 0

Table 1

Treatment Results From Infected Rabbit Corneas[2]

Treatment Group	Staphylococcus aureus	Pseudomonas aeruginosa	
	Log CFU at 10 hours	Log CFU at 20 hours	SLE scores at 20 hours
Untreated	6.8 ± 0.13	7.4 ± 0.07	11.1 ± 0.58^c
Vehicle Control	6.7 ± 0.21	7.4 ± 0.04	11.4 ± 0.68^c
Moxifloxacin – 0.2%	3.8 ± 0.45^a	5.5 ± 0.28^b	11.1 ± 0.67^c
Moxifloxacin – 0.3%	4.1 ± 0.23^a	3.8 ± 0.69^b	11.7 ± 0.53^c
Moxifloxacin – 0.5%	3.9 ± 0.79^a	2.1 ± 0.31^b	11.5 ± 0.39^c

[a]CFU not significantly different from each other ($P \geq 0.17$), but significantly different from the two control treatments ($P \leq 0.0001$)
[b]CFU significantly different from each other ($P \leq 0.03$), as well as the control groups.
[c]SLE scores were not significantly different from each other.

In the case of *S. aureus* infection, the dosing was a single drop topically 9 hours postinfection and corneas harvested at 10 hours postinfection. Significant killing of the *S. aureus* was observed and was comparable to that for CILOXAN (0.3% ciprofloxacin) tested under these conditions.

In the case of *P. aeruginosa* infection, the dosing was a single topical drop every 30 minutes from 16 to 19 hours postinfection and corneas harvested at 20 hours postinfection. A dose dependent killing was observed: 0.3% moxifloxacin was comparable to CILOXAN (0.3% ciprofloxacin) tested under these conditions.

4. REFERENCES

    1. Hobden, J. A., Engel, L. S., Callegan, M. C., Hill, J. M., Gebhardt, B. M., O'Callaghan, R. J. 1993. *Pseudomonas aeruginosa* keratitis in leukopenic rabbits. Curr. Eye Res. 12:461-467.

    2. Laboratory Notebook 7400:045.

Survey of Ophthalmology

Ophthalmology

International Review Journal

The Potency, Ocular Penetration, and Safety of Moxifloxacin (as VIGAMOX® Solution), a Topical Ophthalmic Fourth-Generation Fluoroquinolone

Special Supplement to

Survey of Ophthalmology

The Potency, Ocular Penetration, and Safety of Moxifloxacin (as VIGAMOX® Solution), a Topical Ophthalmic Fourth-Generation Fluoroquinolone

Barry A. Schlech, PhD, Editor

Special Supplement: The Potency, Ocular Penetration, and Safety of Moxifloxacin (as VIGAMOX® Solution), a Topical Ophthalmic Fourth–Generation Fluoroquinolone

Contents

Cover. Conjunctivitis (Printed with permission of Robert D. Gross, MBA, MD).

ELSEVIER

INTRODUCTION

Ophthalmic Infections and Their Anti-infective Challenges

Eduardo Alfonso, MD,[1] and Julie Crider, PhD[2]

[1]Bascom Palmer Eye Institute, University of Miami School of Medicine, Miami, Florida; and [2]Alcon Research, Ltd, Fort Worth, Texas, USA

Abstract. This introduction provides an overview of the succeeding articles contained within this supplement on the new fourth-generation fluoroquinolone antibiotic product, moxifloxacin ophthalmic solution 0.5% (VIGAMOX®, Alcon Laboratories, Inc., Fort Worth, TX). Moxifloxacin was developed specifically to address the increasing incidence of resistance to earlier-generation antibiotic molecules. Structural modifications to the moxifloxacin molecule have decreased the likelihood of the development of resistant organisms. This antibiotic has been shown to possess greater activity than previous-generation molecules against gram-positive bacteria while maintaining excellent potency against gram-negative organisms and nontuberculous (atypical) mycobacteria. Moxifloxacin ophthalmic solution 0.5% exhibits enhanced bioavailability due to a unique molecular structure that combines high lipophilicity for enhanced corneal penetration with high aqueous solubility at physiological pH. Numerous studies have shown that moxifloxacin ophthalmic solution 0.5% has high potency against a broad range of microbial species and a favorable profile in terms of safety and tolerability. The results presented in this supplement provide additional evidence for the potential benefits of moxifloxacin ophthalmic solution 0.5% in surgical prophylaxis and treatment of sight-threatening infections, such as bacterial conjunctivitis, endophthalmitis and keratitis. (Surv Ophthalmol 50:S1–S6, 2005. © 2005 Elsevier Inc. All rights reserved.)

Key words. antibiotic • anti-infectives • moxifloxacin • penetration • potency • safety • therapy • VIGAMOX®

Antibiotic Therapy

This supplement provides clinicians with comprehensive information regarding a new antibiotic therapy for the prevention and treatment of bacterial ocular infections. The focus of this supplement is the new fourth-generation fluoroquinolone antibiotic product, moxifloxacin ophthalmic solution 0.5% (Vigamox, Alcon Laboratories, Inc., Fort Worth, TX), which was recently introduced for the treatment of bacterial conjunctivitis. Preclinical study results from

in vitro and animal experiments as well as results from human clinical trials and postmarket studies will be discussed. These results will provide evidence for the broad utility of moxifloxacin ophthalmic solution 0.5% in the prevention and treatment of a wide variety of ocular infections.

The increasing number of reports concerning ocular bacterial resistance to earlier-generation fluoroquinolones has prompted the development of more advanced fourth-generation antibiotics.[2,5,9]

S1

Moxifloxacin interferes with bacterial deoxyribonucleic acid gyrase (topoisomerase II) and topoisomerase IV, which are enzymes involved in deoxyribonucleic acid replication within the bacteria.[26] Moxifloxacin is more balanced in its inhibition of these two enzymes. Therefore, the likelihood of producing resistant organisms is diminished considerably, because two simultaneous mutations are required to establish bacterial resistance.[12,30,34] In addition, moxifloxacin possesses a unique bicyclic side chain at the C-7 position that inhibits the efflux mechanism of the bacterial cell and results in rapid death of the target microorganism.[26] The advances in the molecular structure of moxifloxacin provide it with greater potency against gram-positive organisms than was seen with earlier-generation fluoroquinolones, while maintaining similar activity against gram-negative bacteria. The issue of potency and efficacy is covered in detail in this supplement in Schlech and Alfonso's article.[28]

The increasing number of ocular surgical procedures is associated with an increasing risk for perioperative infection. Recent reports indicate that the incidence of bacterial infections after cataract and refractive surgery may be rising.[6,15,24] The risk of surgical complications, such as postoperative endophthalmitis and keratitis, underscores the need for potent new-generation antibiotics for the prevention and treatment of these potentially serious ocular infections. Recent guidelines by the Medicare National Surgical Infection Prevention Project indicate the benefits of preoperative and postoperative antibiotic therapy.[4]

Potency and Efficacy of Moxifloxacin Ophthalmic Solution 0.5%

IN VITRO POTENCY

The Endophthalmitis Vitrectomy Study demonstrated that approximately 94% of isolates from postoperative endophthalmitis are gram-positive bacteria.[10] Therefore, a molecule with greater potency against these pathogenic organisms may provide a therapeutic benefit in the prophylaxis of bacterial endophthalmitis. Studies using bacterial isolates have provided useful information regarding the potency of moxifloxacin ophthalmic solution 0.5% against the target bacteria. Mather and colleagues determined the minimum inhibitory concentrations (MICs) for 93 bacterial endophthalmitis isolates for ciprofloxacin, ofloxacin, levofloxacin, gatifloxacin, and moxifloxacin.[21] Overall, moxifloxacin was the most potent fluoroquinolone tested against gram-positive bacteria (P = 0.05). Ciprofloxacin, levofloxacin, gatifloxacin, and moxifloxacin demonstrated

similar potencies against most gram-negative bacteria. In studies with bacterial keratitis isolates, Kowalski et al also reported that moxifloxacin had lower MICs for most gram-positive bacteria than ciprofloxacin, ofloxacin, levofloxacin, or gatifloxacin.[19] These investigators also reported that all fluoroquinolone-susceptible Pseudomonas aeruginosa were 100% susceptible to the five fluoroquinolones tested. Aliprandis and colleagues showed that moxifloxacin ophthalmic solution 0.5% was equivalent to ciprofloxacin 0.3% for P. aeruginosa in an in vivo animal infection model.[3]

Mycobacterium chelonae and Mycobacterium fortuitum are the two most common species of nontuberculous mycobacteria found in bacterial keratitis cases.[8,13] These atypical pathogenic bacteria are being found with increasing frequency in surgical settings.[25] Moxifloxacin exhibits excellent activity against these organisms, with MIC$_{90}$ values of ≤ 1.6 µg/mL and ≤ 1.0 µg/mL for M. chelonae and M. fortuitum, respectively.[1] An antibiotic product, such as moxifloxacin ophthalmic solution 0.5%, with a broad-spectrum coverage of gram-positive and gram-negative organisms that also exhibits potency against atypical mycobacteria may be useful for prevention of postoperative ocular infections.

POTENCY AND EFFICACY IN ANIMAL MODELS

Moxifloxacin ophthalmic solution 0.5% has, for the first time to our knowledge, actually demonstrated potency and efficacy in the prevention of postoperative bacterial endophthalmitis.[19,20] Additionally, in experimental P. aeruginosa and Serratia marcescens rabbit keratitis models, this fluoroquinolone formulation provided significant decreases in colony-forming units.[31] Topical moxifloxacin 0.5% was equivalent in efficacy to vancomycin 50 mg/mL for treating ciprofloxacin-resistant methicillin-resistant Staphylococcus aureus rabbit keratitis.[3] In another study by the same investigators, moxifloxacin (0.5%) showed an efficacy similar to that of ciprofloxacin (0.3%) for the treatment of P. aeruginosa keratitis in rabbits. Moxifloxacin treatment also produced a 5.8-log reduction in colony-forming units per cornea in an experimental nontuberculous mycobacterial keratitis rabbit model (F1). The results of these studies suggest a use for moxifloxacin ophthalmic solution 0.5% in various ophthalmic surgical settings.

F1 Caballero AR, Thibodeaux BA, Dajcs JJ, et al: Effectiveness of fluoroquinolone antibiotics for experimental Mycobacterium chelonae keratitis [abstract]. Presented at the 2003 Meeting of the Ocular Microbiology and Immunology Group; Anaheim, CA; November 15, 2003.

SUCCESSFUL TREATMENT OF OCULAR INFECTIONS IN HUMAN CLINICAL STUDIES

Moxifloxacin ophthalmic solution 0.5% was evaluated in clinical safety and efficacy trials using twice a day (b.i.d.) or three times a day (t.i.d.) dosing regimens across studies in newborns (neonates), infants and toddlers, children, adolescents, and adults. The clinical trials involved nearly 2,000 patients from 2 days to 93 years of age. These studies showed that moxifloxacin ophthalmic solution 0.5% is a successful clinical therapy, curing the cardinal clinical signs of bacterial conjunctivitis (i.e., bulbar conjunctival injection and conjunctival discharge/exudate) at a rate of up to 94%. The five primary ocular prether-apy pathogens isolated in the studies were *Staphylococcus epidermidis*, *S. aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Chlamydia trachomatis*. In all of the studies, moxifloxacin ophthalmic solution 0.5% was safe for and well tolerated by patients of all ages. Moxifloxacin ophthalmic solution 0.5% is an effective therapy for bacterial conjunctivitis that successfully treats the key signs of the disease while effectively eradicating its associated pathogens. (F3)

Ocular Penetration of Moxifloxacin Ophthalmic Solution 0.5%

For successful control of infections in the eye, a topical antibiotic must effectively penetrate the relevant ocular tissues. The avascular nature of the cornea and vitreous, in particular, limits the uptake of antibiotics into these tissues.[32] Moxifloxacin ophthalmic solution 0.5% achieves better penetration into the cornea and other ocular tissues than other fluoroquinolones.[19,29] This enhanced bioavailability is due to the unique molecular structure of moxifloxacin, which combines high lipophilicity for enhanced corneal penetration with high aqueous solubility at physiological pH. The latter property creates a high concentration gradient at the tear film/corneal epithelial interface, providing a driving force for ocular penetration. *In vitro* studies demonstrated higher permeability in Madin-Darby canine kidney cells for moxifloxacin than for ciprofloxacin, norfloxacin, ofloxacin, levofloxacin, lomefloxacin, or gatifloxacin (F2).[27] This permeability was highly correlated with lipophilicity ($R^2 = 0.92$) and corneal permeability ($R^2 = 0.93$), indicating that the Madin-Darby canine kidney cell model is an excellent predictor of corneal penetration.

Numerous *in vivo* studies have been conducted in rabbits to evaluate the penetration characteristics of moxifloxacin ophthalmic solution 0.5%. In a study using excised rabbit corneas, a 3.6-fold higher corneal permeability coefficient was observed for moxifloxacin than for gatifloxacin (F2).[27] In addition, moxifloxacin transversed the cornea twice as fast as gatifloxacin and demonstrated no effect on epithelial tight cell junctions. Another study in rabbits showed that moxifloxacin ophthalmic solution 0.5% was readily absorbed into anterior ocular tissues with concentrations of 12.5, 1.8, and 6.3 µg/g in the cornea, aqueous humor, and iris-ciliary body at 30 minutes, respectively (F4).[27] Additional reports showed corneal concentrations of moxifloxacin that were at least 700-fold above the MIC for fluoroquinolone-susceptible *S. aureus* and at least 19-fold higher than the corresponding MIC values for fluoroquinolone-resistant strains of these organisms.[22] In a 4-day multiple-dosing protocol, moxifloxacin ophthalmic solution 0.5% produced peak concentrations in aqueous humor and vitreous humor higher than those for either ofloxacin or gatifloxacin (F5).[27]

The superior penetration of moxifloxacin ophthalmic solution 0.5% into ocular tissues has been confirmed in studies in humans.[27] In one study, patients who were dosed with moxifloxacin ophthalmic solution 0.5% before undergoing cataract surgery achieved maximal aqueous humor concentrations that were 25- to 30-fold above the median MIC for susceptible *S. aureus* and *S. epidermidis* isolates from clinical cases of endophthalmitis.[16] Patients who were scheduled to undergo vitrectomy surgery were dosed with moxifloxacin ophthalmic solution 0.5% prophylactically to evaluate penetration of the antibiotic into the aqueous humor.[11] Concentrations that far exceeded the MIC_{90} were achieved in the aqueous humor for a broad spectrum of pathogens, including *S. epidermidis*, *S. aureus*, *S. pneumoniae*, *Streptococcus pyogenes*, *Propionibacterium acnes*, *H. influenzae*, *Escherichia coli*, *Bacillus cereus*, *Neisseria gonorrhoeae*, *Proteus mirabilis*, and a number of other organisms. Two other clinical studies showed that aqueous humor antibiotic concentrations were about two- to four-fold higher in cataract patients after topical administration of moxifloxacin 0.5% versus gatifloxacin 0.3%

F2 Rusinko A, May J, Liao J, et al: A study of the enhanced corneal penetration of moxifloxacin [abstract]. Invest Ophthalmol Vis Sci 45:4907, 2004.

F3 Alcon Laboratories, Inc; data on file.

F4 Robertson SM, Sanders M, Jasheway D, et al: Penetration and distribution of moxifloxacin and ofloxacin into ocular tissues and plasma following topical ocular administration to pigmented rabbits [abstract]. Invest Ophthalmol Vis Sci 44:1454, 2003.

F5 Robertson SM, Sanders M, Jasheway D, et al: Absorption and distribution of moxifloxacin, ofloxacin and gatifloxacin into ocular tissues and plasma following topical ocular administration to pigmented rabbits [abstract]. Invest Ophthalmol Vis Sci 45:4906, 2004.

(F6).[18,29] Moxifloxacin, ciprofloxacin, ofloxacin, levofloxacin, and gatifloxacin concentrations have also been determined in human conjunctiva after a single topical administration.[33] The mean moxifloxacin tissue concentration was approximately six- to seven-fold higher than the corresponding values for ciprofloxacin (P = 0.0028). The consistent enhanced penetration of topical moxifloxacin ophthalmic solution 0.5% should provide significant therapeutic benefits over other topical ocular fluoroquinolones.

Safety of Moxifloxacin Ophthalmic Solution 0.5%

Moxifloxacin ophthalmic solution 0.5% was shown to be well preserved and meets as well as exceeds all Food and Drug Administration requirements and United States Pharmacopoeia standards for antimicrobial preservative effectiveness without the need of benzalkonium chloride in the product (F7). *S. aureus, P. aeruginosa, E. coli, Candida albicans,* and *Aspergillus niger* were all reduced to levels below the minimums required. In addition, moxifloxacin ophthalmic solution 0.5% was challenged with additional organisms not required by the United States Pharmacopoeia to measure the preservative effectiveness of the product. Moxifloxacin ophthalmic solution 0.5% was effective against the protozoan *Acanthamoeba,* the atypical bacterium *Nocardia,* and the fungus *Fusarium.* These studies demonstrate that moxifloxacin ophthalmic solution 0.5% as a multidose ophthalmic product can be safely used without the fear of microbial contamination.

Several *in vitro* studies have demonstrated the safety of moxifloxacin ophthalmic solution 0.5%. Yee and colleagues compared the effects of moxifloxacin, levofloxacin, gatifloxacin, and ofloxacin ophthalmic solutions on human corneal epithelial cells *in vitro* (F8).[17] Moxifloxacin exhibited the least amount of cytotoxicity of the antibiotics tested. In studies from the same report of epithelial healing in chickens after PRK, moxifloxacin treatment resulted in significantly smaller wound sizes than levofloxacin 60 and 66 hours after surgery (P < 0.05).

F6   McCulley JP, Surratt G, Shine W: 4th generation fluoroquinolone penetration into aqueous humor in humans [abstract]. Invest Ophthalmol Vis Sci 45:4927, 2004.

F7   Schlech BA, Sutton A, Rosenthal RA, et al: Antimicrobial preservative effectiveness of VIGAMOX [abstract]. Invest Ophthalmol Vis Sci 45:4913, 2004.

F8   Yee RW, Sorour HM, Yee SB, et al: Comparison of relative toxicity of four ophthalmic antibiotics using the human cornea epithelial cell culture system [abstract]. Invest Ophthalmol Vis Sci 45:4939, 2004.

In rabbit studies, lower carboxyfluorescein permeability was observed with moxifloxacin ophthalmic solution 0.5% treatment relative to gatifloxacin ophthalmic solution 0.3% (Zymar®, Allergan, Irvine, CA) (F9). Therefore, moxifloxacin ophthalmic solution 0.5% demonstrated superior maintenance of corneal integrity compared with gatifloxacin ophthalmic solution 0.3%. Using confocal microscopy techniques, Jester and colleagues established a correlation between corneal epithelial thinning (from superficial cells loss) and mild ocular irritation.[14] Confocal microscopy studies performed by Kovoor et al in rabbits evaluated the effects of five topical antibiotic products (0.3% ciprofloxacin, 0.3% ofloxacin, 0.5% levofloxacin, 0.3% gatifloxacin, and 0.5% moxifloxacin) on the epithelial surface of the cornea.[18] All products except moxifloxacin ophthalmic solution 0.5% contained 0.005% or 0.006% benzalkonium chloride. Tears Naturale Free (Alcon Laboratories Inc, Fort Worth, TX) was used as a control. The data showed that 0.5% moxifloxacin did not cause a significant change in corneal epithelial cell layer thickness. After 6 days of treatment, all drug-treated groups, except moxifloxacin ophthalmic solution 0.5% and Tears Naturale Free, caused significant thinning of the corneal epithelial layer. In all groups, the corneal stromal thickness was similar to baseline. Conversely, 0.3% ciprofloxacin, 0.3% ofloxacin, 0.5% levofloxacin, and 0.3% gatifloxacin caused significant thinning of the corneal epithelial layer after 7 days of antibiotic treatment.

A subchronic ophthalmic safety study in cynomolgus monkeys used a dosing regimen of 2 drops, six times a day for 16 days, followed by t.i.d. dosing for the remainder of the 3-month study. Treatments were 0% (vehicle), 0.5%, 1.0%, and 3.0% moxifloxacin ophthalmic solutions. No significant findings were observed for any systemic parameters that were measured (F10).[23] Indirect ophthalmoscopic and slit-lamp biomicroscopic examinations were similar for controls and moxifloxacin treated groups. IOP and specular microscopy revealed no findings related to moxifloxacin treatment. In addition, corneal thickness, a sensitive indicator of corneal health, was not affected by administration of moxifloxacin ophthalmic solution, even at the highest concentrations and most extreme regimens.

F9   Owen GR, Dembinska O, Stout KR, Mendiola MK: Corneal penetration and change in corneal permeability of moxifloxacin versus gatifloxacin [abstract]. Invest Ophthalmol Vis Sci 45:4910, 2004.

F10   Bergamini MVW, Heaton J, McGee D, et al: A three month topical ocular toxicity study of moxifloxacin ophthalmic solutions in cynomolgus monkeys [abstract]. Invest Ophthalmol Vis Sci 44:4457, 2003.

General-safety pharmacology studies were designed to profile effects of moxifloxacin hydrochloride on all major organ systems. Moxifloxacin hydrochloride produced no overt side effects in the central nervous systems of test animals (rats and mice) at the highest intravenous dose tested (i.e., 30 mg/kg), which is 1,000-fold higher than the maximum daily dose of moxifloxacin ophthalmic solution 0.5%.[23] In conclusion, the safety margin determined in all of the safety pharmacology studies provides strong support that moxifloxacin hydrochloride administered by the topical ocular route is unlikely to promote significant adverse events in human beings.

Human studies have also confirmed the safety and tolerability of moxifloxacin ophthalmic solution 0.5%. Confocal microscopy experiments reported no changes in the number or morphology of corneal epithelium and endothelium for normal patients treated with moxifloxacin ophthalmic solution 0.5% four times daily for 3 days (F11). In cataract surgery patients, Katz et al evaluated the ocular absorption of moxifloxacin ophthalmic solution 0.5% by measuring the concentration of moxifloxacin in the aqueous humor of patients undergoing cataract surgery.[16] The authors also noted that the administration of moxifloxacin ophthalmic solution 0.5% before cataract surgery had no effect on postoperative corneal and conjunctival healing.

Yee and colleagues evaluated the effects of topical moxifloxacin 0.5% ophthalmic solution and gatifloxacin 0.3% ophthalmic solution on corneal wound healing for patients undergoing bilateral PRK.[35] These authors concluded that both products were safe in PRK. Moxifloxacin ophthalmic solution 0.5% and gatifloxacin ophthalmic solution 0.3% produced statistically identical results with respect to haze, visual acuity, and rate of corneal wound healing when administered to PRK patients preoperatively and postoperatively.

Durrie and Trattler showed that moxifloxacin ophthalmic solution 0.5% and gatifloxacin ophthalmic solution 0.3% were equivalent with respect to all ophthalmologic measures, the quality of vision, and comfort for patients who had undergone laser-assisted *in situ* keratomileusis and laser epithelial keratomileusis surgery.[7] In addition, moxifloxacin ophthalmic solution 0.5% was shown to be as comfortable as a tear substitute in pediatric subjects (F12). In summary,

moxifloxacin ophthalmic solution 0.5% exhibits a desirable safety profile that is an important factor in the prevention and treatment of bacterial infections of an ocular surface that may be compromised to various degrees after surgical procedures.

The Future of Ophthalmic Therapy and the Role of Moxifloxacin Ophthalmic Solution 0.5%

Moxifloxacin ophthalmic solution 0.5% represents a major advance in the treatment and prevention of ocular infections. The increased resistance to the earlier-generation fluoroquinolones (primarily from human systemic and animal husbandry use) prompted the development of the new fourth-generation molecules, such as moxifloxacin, in moxifloxacin ophthalmic solution 0.5%. Moxifloxacin possesses a unique molecule structure that provides superior potency, penetration, and safety. Taken together, these strengths make moxifloxacin ophthalmic solution 0.5% a valuable addition to ophthalmologists' armamentarium of antibiotics for the prevention and treatment of ocular infections. This fourth-generation fluoroquinolone is currently approved in the USA, Canada and India for the treatment of bacterial conjunctivitis. The results presented in this supplement provide additional evidence for the potential benefits of moxifloxacin ophthalmic solution 0.5% in surgical prophylaxis and treatment of sight-threatening infections such as bacterial conjunctivitis, keratitis, and endophthalmitis.

Method of Literature Search

We performed a literature search for this article based on MEDLINE database searches from 1990 to 2005, using various combinations of the search terms *ocular infections, ophthalmic infections, ophthalmic antibiotics, fluoroquinolones, Vigamox, Zymar, moxifloxacin, gatifloxacin, therapy,* and *prophylaxis*. Relevant English journal articles and/or abstracts were selected for review.

References

1. Abshire R, Cockrum P, Crider J, Schlech B: Topical antibacterial therapy for mycobacterial keratitis: potential for surgical prophylaxis and treatment. Clin Ther 26:191–6, 2005
2. Alexandrakis G, Alfonso EC, Miller D: Shifting trends in bacterial keratitis in South Florida and emerging resistance to fluoroquinolones. Ophthalmology 107:1497–502, 2000
3. Aliprandis E, Ciralsky J, Lai H, et al: Comparative efficacy of topical moxifloxacin versus ciprofloxacin and vancomycin in the treatment of *P. aeruginosa* and ciprofloxacin-resistant MRSA keratitis in rabbits. Cornea 24:201–5, 2005
4. Bratzler DW, Houck PM: Surgical Infection Prevention Guidelines Writers Workgroup: Antimicrobial prophylaxis for surgery: an advisory statement from the National Surgical

---

F11 Donaldson KE, Marangon FB, Schatz L, et al: Confocal analysis of the effects of moxifloxacin on the normal human cornea [abstract]. Am Soc Cataract Refract Surg, 2004.

F12 Wagner RS, D'Arienzo PA, Hallas SJ, et al: A comparative study in a normal pediatric population of the relative comfort of moxifloxacin 0.5% ophthalmic solution versus a tear substitute [abstract]. Invest Ophthalmol Vis Sci 45:4936, 2004.

Infection Prevention Project. Clin Infect Dis 38:1706–15, 2004

5. Chaudhry NA, Flynn HW Jr, Murray TG, et al: Emerging ciprofloxacin-resistant *Pseudomonas aeruginosa*. Am J Ophthalmol 128:509–10, 1999

6. Colleaux KM, Hamilton WK: Effect of prophylactic antibiotics and incision type on the incidence of endophthalmitis after cataract surgery. Can J Ophthalmol 35:373–8, 2000

7. Durrie DS, Trattler W: A comparison of therapeutic regimens containing moxifloxacin 0.5% ophthalmic solution and gatifloxacin 0.3% ophthalmic solution for surgical prophylaxis in patients undergoing LASIK or LASEK. J Ocular Pharm Ther 1:236–41, 2005

8. Ford JG, Huang AJW, Pflugfelder SC, et al: Nontuberculous mycobacterial keratitis in South Florida. Ophthalmology 105:1652–8, 1998

9. Goldstein MH, Kowalski RP, Gordon YJ: Emerging fluoroquinolone resistance in bacterial keratitis: five-year review. Ophthalmology 106:1313–8, 1999

10. Han DP, Wisniewski SR, Wilson LA, et al: Spectrum and susceptibilities of microbiologic isolates in the Endophthalmitis Vitrectomy Study. Am J Ophthalmol 122:1–17, 1996

11. Hariprasad SM, Blinder KJ, Shah GK, et al: Penetration pharmacokinetics of topically administered moxifloxacin 0.5% ophthalmic solution in human aqueous and vitreous Arch Ophthalmol 123:39–44, 2005

12. Hooper DC: Fluoroquinolone resistance among gram-positive cocci. Lancet Infect Dis 2:530–8, 2002

13. Huang SCM, Soong HK, Chang J-S, Liang Y-S: Non-tuberculous mycobacterial keratitis: a study of 22 cases. Br J Ophthalmol 80:962–8, 1996

14. Jester JV, Maurer JK, Petroll WM, et al: Application of in vivo confocal microscopy to the understanding of surfactant-induced ocular irritation. Toxicol Pathol 24:412–28, 1996

15. Karp CL, Tuli SS, Yoo SH, et al: Infectious keratitis after LASIK. Ophthalmology 110:503–10, 2003

16. Katz HR, Masket S, Lane S, et al: Absorption of topical moxifloxacin ophthalmic solution into human aqueous humor. Ophthalmology (In press)

17. Kim DH, Stark WJ, O'Brien TP, Dick JD: aqueous penetration and biological activity of moxifloxacin 0.5% ophthalmic solution and gatifloxacin 0.3% solution in cataract surgery patients. Cornea (In press)

18. Kovoor TA, Kim AS, McCulley JP, et al: Evaluation of the corneal effects of topical ophthalmic fluoroquinolones using in vivo confocal microscopy. Eye Contact Lens 30:90–4, 2004

19. Kowalski RP, Dhaliwal DK, Karenchak LM, et al: Gatifloxacin and moxifloxacin: an in vitro susceptibility comparison to levofloxacin, ciprofloxacin, and ofloxacin using bacterial keratitis isolates. Am J Ophthalmol 136:500–5, 2003

20. Kowalski RP, Romanowski EG, Mah FS, et al: Topical prophylaxis with moxifloxacin prevents endophthalmitis in a rabbit model. Am J Ophthalmol 138:33–7, 2004

21. Mather R, Karenchak LM, Romanowski EQ, Kowalski RP: Fourth generation fluoroquinolones: new weapons in the arsenal of ophthalmic antibiotics. Am J Ophthalmol 133:463–6, 2002

22. Mather R, Stewart J, Prabriputaloong T, et al: The effect of cataract surgery on ocular levels of topical moxifloxacin. Am J Ophthalmol 138:554–9, 2004

23. McGee DH, Holt WF, Kastner PR, Rice RL: Safety of moxifloxacin as shown in animal and in vitro studies. Surv Ophthalmol (In press)

24. Nagaki Y, Hayasaka S, Kadoi C, et al: Bacterial endophthalmitis after small-incision cataract surgery: effect of incision placement and intraocular lens type. J Cataract Refract Surg 29:20–6, 2003

25. Newman PE, Goodman RA, Waring GO, et al: A cluster of cases of *Mycobacterium chelonei* keratitis associated with outpatient office procedures. Am J Ophthalmol 97:344–8, 1984

26. Pestova E, Millichap JJ, Noskin GA, Peterson LR: Intracellular targets of moxifloxacin: a comparison with other fluoroquinolones. J Antimicrob Chemother 45:583–90, 2000

27. Robertson SM, Curtis MA, Schlech BA, et al: Ocular pharmacokinetics of moxifloxacin after topical treatment of animals and humans. Surv Ophthalmol 50 (Suppl 1:S32–S45, 2005

28. Schlech BA, Alfonso E: Overview of the potency of moxifloxacin ophthalmic solution 0.5% (VIGAMOX®). Surv Ophthalmol 50 (Suppl) 1:S7–S15, 2005

29. Solomon R, Donnenfeld ED, Perry HD, et al: Penetration of topically applied gatifloxacin 0.3%, moxifloxacin 0.5%, and ciprofloxacin 0.3% into the aqueous humor. Ophthalmology 112:466–9, 2005

30. Tanaka M, Wang T, Onodera Y, et al: Mechanisms of quinolone resistance in *Staphylococcus aureus*. J Infect Chemother 6:131–9, 2000

31. Thibodeaux BA, Dajcs JJ, Caballero AR, et al: Quantitative comparison of fluoroquinolone therapies of experimental gram-negative bacterial keratitis. Curr Eye Res 28:337–42, 2004

32. Thielen TL, Castle SS, Terry JE: Anterior ocular infections: an overview of pathophysiology and treatment. Ann Pharmacother 34:235–46, 2000

33. Wagner RS, Abelson MB, Shapiro A, Torkildsen G: Evaluation of moxifloxacin, ciprofloxacin, gatifloxacin, ofloxacin, and levofloxacin concentrations in human conjunctival tissue. Arch Ophthalmol 123:1282–3, 2005

34. Wang MG, Tran JH, Jacoby GA, et al: Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai China. Antimicrob Agents Chemother 47:2742–8, 2003

35. Yee RW, Setabutr P, Foltermann et al.: The effects of topical moxifloxacin 0.5% ophthalmic solution and galifloxacin 0.3% solution on corneal healing following bilateral photorefractive keratectomy (PRK). Cornea (In press)

ELSEVIER

Overview of the Potency of Moxifloxacin Ophthalmic Solution 0.5% (VIGAMOX®)

Barry A. Schlech, PhD,[1] and Eduardo Alfonso, MD[2]

[1]Alcon Research, Ltd., Fort Worth, Texas; and [2]Bascom Palmer Eye Institute, University of Miami School of Medicine, Miami, Florida, USA

Abstract. Antibiotics have been the mainstay of therapy for infectious diseases since their origins in the 1940s. As microorganisms changed and resistance developed, more advanced antibiotics were ultimately needed to provide adequate coverage and spectrum. By selecting optimal antibiotics and dosing regimens, clinicians can avoid treatment failures and adverse events and can help prevent the emergence of further antibiotic resistance. The fourth-generation ophthalmic fluoroquinolones include moxifloxacin (VIGAMOX®, Alcon Laboratories, Inc., Fort Worth, TX) and gatifloxacin (Zymar, Allergan, Irvine, CA), and they are now approved for the treatment of bacterial conjunctivitis. This review highlights four scientific methods that compare and rank antibiotic potencies and predict their clinical efficacy and their propensity to develop resistance: 1) in vitro assay for minimum inhibitory concentrations, 2) in vivo models for pharmacokinetic and pharamacodynamic properties, 3) therapeutic index or inhibitory quotient, and 4) in vitro assay for mutant prevention concentration. The fourth-generation ophthalmic fluoroquinolones perform well in these assays. Both antibiotics have better in vitro activity against gram-positive bacteria than ciprofloxacin or ofloxacin. Moxifloxacin penetrates better into ocular tissues than gatifloxacin and older fluoroquinolones; in vitro activity of moxifloxacin and gatifloxacin against gram-negative bacteria is similar to that of older fluoroquinolones. Moxifloxacin also has better mutant prevention characteristics than other fluoroquinolones. These findings support the use of the newer fluoroquinolones for the prevention and treatment of serious ophthalmic infections (e.g., keratitis, endophthalmitis) caused by susceptible bacteria. (Surv Ophthalmol 50:S7–S15, 2005. © 2005 Elsevier Inc. All rights reserved.)

Key words. antibiotic • breakpoints • fluoroquinolone • in vitro • in vivo • MIC • moxifloxacin • pharmacodynamics • pharmacokinetic • potency • MPC • therapeutic index • VIGAMOX®

Since the 1940s, antibiotics have been the mainstay of therapy for infectious diseases. Newer antibiotics with different molecular structures were ultimately needed to overcome the resistance developed by the microorganisms to earlier antimicrobial compounds. For ophthalmology, the story is much the same. With the emergence of antibiotic-resistant organisms, it is essential that clinicians prescribe antibiotics and dosing regimens that will effectively prevent and treat specific sight-threatening ocular infections, such as endophthalmitis and keratitis. To do so, they need to know which antibiotics are most effective against common ocular pathogens. By selecting optimal antibiotics and dosing regimens, clinicians can avoid

S7

treatment failures and adverse events and can help to prevent the emergence of further antibiotic resistance.

The New Fluoroquinolones

The fourth-generation ophthalmic fluoroquinolones include moxifloxacin (VIGAMOX®, Alcon Laboratories, Inc. Fort Worth, TX) and gatifloxacin (Zymar®, Allergan, Irvine, CA), and they are now approved for the treatment of bacterial conjunctivitis. These new fluoroquinolones are being used off-label by ophthalmologists to prevent bacterial endophthalmitis in patients undergoing surgery.[1,20,28,41] These agents are also commonly being used off-label to treat bacterial keratitis.[24] These new agents provide better coverage against gram-positive organisms and atypical mycobacteria than previously available antibiotics; are more potent, especially against resistant gram-positive pathogens; and delay the emergence of antibiotic-resistant pathogens (F1).[6,23]

Predicting Fluoroquinolone Efficacy

We are now seeing the results of a number of clinical trials that directly compare the efficacies of the new fluoroquinolones to those of older generations of drugs for topical ophthalmic use.[35,42] Such trials guide clinicians in evaluating their relative potency and help them determine which product to recommend for patients. However, there are four scientific methods available to clinicians to help them compare and rank antibiotic potencies of fluoroquinolones and predict their clinical efficacy or their propensity for resistance development.

IN VITRO ASSAY OF MINIMUM INHIBITORY CONCENTRATIONS (MICs)

The MIC involves an *in vitro* determination of antibiotic potency against specific pathogens by comparing the inhibitory activity of various concentrations of a drug against a known inoculum of bacteria (e.g., 10^5 colony-forming units/mL).[4] Other *in vitro* assays assess the killing capability of antibiotics against particular organisms. For example, Stroman and colleagues (F2) conducted *in vitro* studies using clinical isolates of *S. pneumoniae* obtained from patients with bacterial conjunctivitis. Moxifloxacin demonstrated faster kinetics of kill for *S. pneumoniae* than either tobramycin (Tobrex®, Alcon Laboratories, Inc., Fort Worth, TX) gentamicin (Genoptic®,

---

F1 Pharmaceutical Association. New Product Bulletin: Avelox™ (moxifloxacin HCL). Washington, 2000.

F2 Stroman et al unpublished data, 2005.

Allergan, Inc., Irvine, CA), or trimethoprim/polymyxin (Polytrim®, Allergan). These *in vitro* data suggest that the faster kill provided by moxifloxacin prevents the spread of infection and therefore decreases the contagiousness of bacterial conjunctivitis.

IN VIVO PHARMACOKINETIC AND PHARMACODYNAMIC MODELS

These models evaluate the penetration and distribution of a drug via one mode of administration into ocular tissues. They consider the *in vitro* MIC of an antibiotic with the drug's *in vivo* pharmacokinetic properties to more accurately predict clinical cures.[22] For example, Wagner and colleagues determined the concentrations of several fluoroquinolones in human conjunctival tissue.[35,42] Twenty minutes following instillation of a single dose, the conjunctival tissues concentrations (µg/g) were: moxifloxacin (18.0), gatifloxacin (2.54), ofloxacin (1.26), ciprofloxacin (2.65) and levofloxacin (2.34). Moxifloxacin demonstrated significantly ($P < 0.0001$) greater penetration to the target tissue than did the other fluoroquinolones.

THERAPEUTIC INDEX OR INHIBITORY QUOTIENT

This index combines MIC data with *in vivo* concentration data at the actual site of infection in tissues and fluids and is, therefore, potentially the most accurate predictor of *in vivo* antibiotic efficacy.[27] Several studies have used MICs and pharmacodynamic models to compare and contrast the potency of the fourth-generation fluoroquinolones with that of earlier generations.[10,20]

IN VITRO ASSAY FOR MUTANT PREVENTION CONCENTRATION (MPC)

The MPC is a novel *in vitro* measurement that determines the propensity of an antimicrobial compound to select for antimicrobial-resistant subpopulations when high-density bacterial inocula (e.g., $>10^9$ organisms) are exposed to various drug concentrations. In human infectious diseases, the number of organisms present at the site of infection is large and may exceed the spontaneous mutational frequency rate, and as such, there may be substantial resistant subpopulations present in high-density cultures. This technology has been applied mostly to the fluoroquinolones and gram-positive organisms. Because resistance to fluoroquinolones occurs in a stepwise fashion, with a single mutation being a first-step resistant mutant and a second mutation representing a second-step resistant mutant, the MPC approach defines the drug concentration required to block the growth of organisms containing first-step resistance mutations. As such, MPC testing is relevant

only with organisms that are determined to be susceptible to the antimicrobial agent by traditional susceptibility testing.[7]

The following discussion is intended to assist ophthalmologists in understanding these four methods of evaluating antibiotic potency, interpreting data from studies using these methods, and applying study results in their clinical practices.

MICs and MPCs: Evaluation of *In Vitro* Antimicrobial Potency and Antibiotic Resistance Development

The MIC is an *in vitro* determination of the lowest concentration of a specific antibiotic that inhibits the growth of an organism at a defined inoculum of bacteria (usually 10^5 colony-forming units/mL).[4] MPCs are another measure of potency and reflect on the antibiotic's robustness in preventing mutant or resistant strain development.[7,25]

DETERMINING MICs

The MIC of an antibiotic for a particular strain of bacteria is determined by standardized microbiological agar and broth tests. The MIC_{90} represents the antibiotic concentration that inhibits 90% of the isolates tested and is calculated when there are at least 10 isolates of a particular microorganism. Likewise, the MIC_{50} represents the antibiotic concentration that inhibits 50% of the isolates tested and can be calculated when there are at least five isolates. In MIC tests, surviving microorganisms are detected by their ability to produce visible growth either on a series of agar plates (i.e., agar dilution or agar diffusion methods) or in microtiter plate wells of broth (i.e., microbroth dilution tests).

Agar Dilution Method

In the agar dilution method, Petri dishes are filled with growth media containing various concentrations of antibiotic and solidified with agar. A defined amount of test organism is inoculated onto the top surface of the solid medium. After incubation, at the proper temperature and atmosphere, for approximately 18–24 hours, the plates are screened for growth. The lowest drug concentration preventing growth is the MIC.

Agar Diffusion Methods

Two other agar-based tests use agar that does not have the antibiotic incorporated into the media. For the Kirby-Bauer (disk diffusion) test, an antibiotic impregnated paper disk is placed on top of the agar that has been inoculated with bacteria. Alternatively, a paper strip containing increasing concentrations

of antibiotic is placed on top of the seeded agar (an E test). For both Kirby-Bauer and E tests, the agar plates are incubated overnight to allow the bacterial inoculum to grow and form a continuous thin film of growth on top of the agar, except where growth of the bacterial isolate is inhibited by the antibiotic. This visible zone of inhibition around the disk is measured, and the zone size (Kirby-Bauer) is used to estimate the relative susceptibility or resistance of the organism to the antibiotic. The E test yields an actual MIC. The bacterial strain is reported as sensitive, intermediate, or resistant, depending on the zone size or the MIC.

Broth Dilution Methods

Similar antibiotic dilutions and microorganism challenges are performed using microbiological broth in test tubes or microtiter plates containing various concentrations of antibiotic. Bacterial growth or no growth is measured after incubation and MICs are determined.

Semiautomated instruments may also be used to determine an organism's susceptibility to an antimicrobial compound. The antibiotic with the lowest MIC, MIC_{90}, or MIC_{50} for a particular bacteria is more potent than those antibiotics with higher MICs.[24] However, the *in vitro* MIC data need to be considered along with achievable and sustainable drug concentrations at the site of infection (i.e., various pharmacokinetic and pharmacodynamic parameters). The true killing potential of an antibiotic can be determined in time kill studies, because MICs are a measure of inhibition of bacterial growth. Theoretically, however, the more potent an antibiotic, the less likely the antibiotic will be present at sublethal concentrations and result in partial bacterial killing and, thus, possibly induce resistance.[24] To ensure the therapeutic efficacy of an antibiotic *in situ*, it is necessary to maintain its concentration above the MIC. For concentration-dependent antibiotics, like moxifloxacin, this desired concentration is usually at least 8–10 times the MIC. For concentration-independent or time-dependent antibiotics, like vancomycin, it is the time above the MIC that is more important.

ANTIBIOTIC BREAKPOINTS

The primary function of *in vitro* antimicrobial susceptibility testing in clinical diagnostic laboratories is to provide information to clinicians to guide their selection of antibiotics for therapy. Antibiotic susceptibility or MIC testing is used in clinical research to determine the *in vitro* activity of new antibiotics and to track the incidence and prevalence of antimicrobial resistance.[22] The data from MIC testing are the basis for generating standardized breakpoints (i.e.,

cutoff values) that categorize particular organisms as susceptible, intermediate, or resistant to specific antibiotics. Although somewhat arbitrary, the MIC breakpoints reflect the safe, achievable plasma or serum concentrations of antibiotics and have been established by the National Committee for Clinical Laboratory Standards,[26] Food and Drug Administration, European Medicines Evaluation Agency,[18] and other groups, such as the International Society of Anti-infective Pharmacology. An organism is defined as susceptible to an antibiotic if its MIC is below the serum breakpoint. Infections caused by that particular organism may be effectively treated by standard doses of the antibiotic, which typically achieve concentrations many times the MIC value in the serum.[8,9,24] Organisms demonstrate intermediate resistance if the MIC falls between the susceptible and resistant breakpoints. Infections caused by these pathogens may, in some cases, be effectively treated with higher antibiotic doses that achieve higher serum and tissue concentrations. Pathogens are considered resistant to the antibiotic if the MIC is at or above the established breakpoint and if infections may not be appropriately treated by even achievable concentrations of that antibiotic at that site.[16] For an organism isolated from a particular infection to be susceptible to oral formulations of gatifloxacin, levofloxacin, moxifloxacin, or ofloxacin, the drug must have an MIC of 2 µg/mL or less. However, to be classified as susceptible to ciprofloxacin, the organism must demonstrate a ciprofloxacin MIC of 1 µg/mL or less. Each genus of organism (e.g., Staphylococcus, Streptococcus) may have different breakpoints. The antibiotic must be present in the affected tissue at concentrations that are usually many times the MIC for a particular organism.[8,9,24] Again, these antibiotic breakpoints were determined based on the concentration of the antibiotic found in the serum of patients on parenteral or oral therapy. Their direct relevance to topically applied antibiotics in ophthalmology is uncertain, but they represent references for ophthalmologists in using antibiotic preparations in their practices.

Some antibiotics do not have assigned breakpoints for every organism. For example, ciprofloxacin does not have an assigned breakpoint for Streptococcus pneumoniae.

COMPARING THE POTENCIES OF FLUOROQUINOLONES

Two recent studies[20,24] determined MIC values for second- (ciprofloxacin and ofloxacin), third- (levofloxacin), and fourth-generation (moxifloxacin and gatifloxacin) fluoroquinolones against endophthalmitis and keratitis isolates and used these values to rank their potencies. Mather et al[24] determined the median MICs for 93 bacterial endophthalmitis isolates using the E-test approach. The antibiotic susceptibility of each infectious isolate was determined by comparing the MIC of each antibiotic to National Committee for Clinical Laboratory Standards for each fluoroquinolone. It should be noted that fewer than 10 organisms were tested for some of the genera/species reported in this study. This is why a median MIC calculation (i.e., MIC$_{50}$) is the most appropriate method for assessing potency in this particular study. As previously stated, susceptibility to ciprofloxacin is indicated by an MIC of 1 µg/mL or less for most organisms, whereas susceptibility to gatifloxacin, levofloxacin, moxifloxacin, or ofloxacin is indicated by an MIC of 2 µg/mL or less. The results of the study demonstrated that strains of Staphylococcus aureus resistant to second-generation fluoroquinolones were more susceptible to moxifloxacin than to gatifloxacin and levofloxacin (P = 0.01). Based on these MIC determinations, the potencies of ciprofloxacin, gatifloxacin, levofloxacin, and moxifloxacin were ranked against gram-positive and gram-negative bacteria, including strains resistant to the older fluoroquinolones.

Compared with the second- and third-generation agents, both moxifloxacin and gatifloxacin had superior MIC activity against fluoroquinolone-susceptible and fluoroquinolone-resistant gram-positive bacterial strains isolated from endophthalmitis cases. Ciprofloxacin and levofloxacin were equally potent against gram-positive bacteria. With the exception of two isolates, ciprofloxacin and levofloxacin were more potent than ofloxacin. In comparing the median MICs for the two fourth-generation agents, moxifloxacin was more potent than gatifloxacin against fluoroquinolone-resistant and fluoroquinolone-susceptible S. aureus as well as fluoroquinolone-susceptible coagulase-negative staphylococci, Streptococcus species (including S. pneumoniae and S. viridans), and Enterococcus species. Moxifloxacin and gatifloxacin were equally potent against fluoroquinolone-resistant coagulase-negative Staphylococcus and Bacillus species.

In studies by Kowalski et al.[20] and Hwang,[15] both median MICs and MIC$_{90}$s of conjunctivitis and keratitis isolates were determined and the potencies of fluoroquinolones were ranked against sensitive and resistant gram-positive and gram-negative organisms. Moxifloxacin and gatifloxacin demonstrated lower MIC$_{90}$s than ciprofloxacin, levofloxacin, or ofloxacin against gram-positive bacteria (MIC ranges of 0.03–2 µg/mL for moxifloxacin/gatifloxacin vs. 0.19–32 µg/mL for ciprofloxacin/ofloxacin/levofloxacin).[15,20] Moxifloxacin and gatifloxacin also demonstrated better in vitro susceptibility for isolates

resistant to second- and third-generation fluoroquinolones and equal susceptibility for gram-negative organisms when compared with the older-generation agents, with the exception of *Pseudomonas aeruginosa*. When the *in vitro* activities of the newer-generation agents were compared, moxifloxacin demonstrated statistically better activity (i.e., lower MIC) than gatifloxacin against fluoroquinolone-resistant strains of *S. aureus*, and gatifloxacin demonstrated lower MICs against fluoroquinolone-susceptible isolates of some gram-negative bacteria (*P. aeruginosa, Moraxella, Haemophilus*) (F3). A Study by Stroman et al[20] described the bacteria isolated from bacterial conjunctivitis cases in the USA, Europe and India and determined the antibiotic susceptibility patterns of over 2,300 isolates. They found that resistance to the fourth-generation fluoroquinolones was very low in all three regions for *Staphylococcus epidermidis* isolates (F4).

The apparent weakness of these antibiotics against *P. aeruginosa* is controversial. Through traditional *in vitro* susceptibility studies, moxifloxacin has been found to be less effective than ciprofloxacin against *P. aeruginosa*.[34,40] Nevertheless, Kowalski et al showed that, although there are differences in MICs, all fluoroquinolone-susceptible *P. aeruginosa* were 100% susceptible to the five tested.[20] Also, in rabbit keratitis studies with *P. aeruginosa*, Aliprandis et al showed that moxifloxacin ophthalmic solution 0.5% was equal to ciprofloxacin ophthalmic solution 0.3% (the gold standard for anti-*Pseudomonas* activity).[2] In addition, Dalhoff generally recognized that ciprofloxacin and ofloxacin are highly active against aerobic or facultative gram-negative bacilli. These fluoroquinolones have concentration-dependent killing rates for gram-negative organisms. Earlier fluoroquinolones are not as active against gram-positive bacteria as they are against gram-negative bacteria. Newer fluoroquinolones, such as moxifloxacin, have enhanced activity against gram-positive bacteria but maintain their activity against gram-negative bacteria.[10]

Fourth-generation fluoroquinolones have a broader spectrum of activity because their molecular structures differ from those of older fluoroquinolones. The molecular structures of moxifloxacin and gatifloxacin have greater binding affinity for and thus inhibit two of the enzymes necessary for bacterial deoxyribonucleic acid synthesis (deoxyribonucleic acid gyrase [also called topoisomerase II] and topoisomerase IV) in both gram-negative and gram-positive microorganisms.[19,29] The older fluoroquinolones adequately inhibit deoxyribonucleic acid gyrase in gram-negative organisms but are not as effective as fourth-generation agents for inhibiting topoisomerase IV in gram-positive organisms.[6] The specificity of this mechanism has important implications in the development of resistance to older fluoroquinolones and in ranking the efficacy of newer fluoroquinolones against resistant strains. Data from trials in resistant mutant species suggest that the newer, fourth-generation fluoroquinolones, such as moxifloxacin and gatifloxacin, have a dual-binding mechanism of action, inhibiting both deoxyribonucleic acid gyrase and topoisomerase IV, in gram-positive species. Owing to the rarity of double mutations (e.g., 10^{-14} for fluoroquinolones in *S. pneumoniae*), the preferential use of such agents could limit the emergence of fluoroquinolone resistance.[5] Another indicator of potency, the MPC, can be used to measure the propensity of an antibiotic to encourage resistance development and can help rank antibiotic potency. Using the MPC approach, Blondeau et al found that for clinical isolates of *S. pneumoniae*, the rank order of antibiotic potencies was moxifloxacin > gatifloxacin > levofloxacin, and for methicillin susceptible *S. aureus*, it was moxifloxacin > gatifloxacin = levofloxacin.[7]

In addition, because of its unique chemical structure, moxifloxacin is a poor substrate for the efflux pump in *S. aureus*.[17,31] The addition of an azabicycloamine side chain on the moxifloxacin molecule makes it more difficult to pump moxifloxacin out of the bacterial cell. This means that once moxifloxacin gets into the bacteria to do its damage, the bacteria has a difficult time pumping out the antibiotic. For a bacterium to resist the lethal effects of moxifloxacin, it must develop more than two mutations or changes to prevent disruption of its two key enzymes, recognize moxifloxacin as a substrate for its efflux mechanism and efficiently pump the antibiotic out of the cell.

LIMITATIONS OF MIC BREAKPOINTS FOR OPHTHALMOLOGY

Although MICs are important *in vitro* indicators of antibiotic activity or potency, they do not necessarily predict *in vivo* antibiotic efficacy. The clinical efficacy of an antibiotic depends on the MIC of the drug relative to the concentration it achieves in target tissues.[21] For example, two antibiotics with an MIC of 1 μg/mL may have different antibacterial effects if one has a peak tissue concentration of 2 μg/mL

F3 Metzler K, Hedlin P, Blondeau J: Determination of minimal inhibitory concentration (MIC) and mutant prevention concentration (MPC) of ocular isolated of *Pseudomonas aeruginosa* (PA) and *Haemophilus influenzae* (HI) to 5 fluoroquinolone (FQ) antimicrobial agents (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4988, 2004.

F4 Stroman DW, Cupp GA, Schlech BA: Microbiology of bacterial conjunctivitis (1999–2003). Invest Ophthalmol Vis Sci 46:E.Abstract 5066, 2005.

and the other a peak tissue concentration of 20 μg/mL.[30] As such, it is necessary to compare *in vitro* microbiological measurements with various pharmacological parameters to fully understand and rank antimicrobial agents and their true potencies.

Pharmacokinetics and Pharmacodynamics

For an antibiotic to achieve optimal efficacy, it must reach the pathogen in the infected tissue and remain there for sufficient time at a concentration required for bacterial killing. The pharmacokinetic and pharmacodynamic properties of each antibiotic determine the *in vivo* relationship between the drug and the pathogen necessary to achieve optimal antibiotic efficacy. For ophthalmology, these parameters determine how well the antibiotic penetrates ocular tissues like the cornea, the conjunctiva, or the aqueous humor.

Pharmacokinetics involves the absorption, distribution, and elimination of a drug. An antibiotic's pharmacokinetic properties include measurements of the antibiotic concentrations achieved in a patient's serum or, in the case of ophthalmology, in the ocular tissues. Several parameters can help do this: 1) area under the concentration curve, 2) peak concentration achieved in the tissue (C_{max}), 3) time to maximum concentration in the tissue, 4) elimination half-life, and 5) penetration at different sites of infection. The area under the concentration of a drug is defined as a measure of how much antibiotic reaches the target tissue throughout a set period (usually 24 hours) and helps estimate the drug's bioavailability. When combined with the drug's dosing regimen, pharmacokinetic properties determine the effective time course of drug concentration into ocular tissues. Fluoroquinolones vary in their pharmacokinetic properties.[30,37,38]

Pharmacodynamics describes the relationship between the concentration of a drug over time at the site of infection and the pharmacologic and toxicologic effects of the drug.[13,43] For antibiotics, pharmacodynamic activity depends on the mechanism of action against the bacteria (i.e., bacterial inhibition or killing) and can be described as being either time- or concentration-dependent.[30] For time-dependent antibiotics, such as vancomycin, β-lactams, and macrolides, bacterial killing depends on the amount of time the drug concentration in the ocular tissues exceeds the MIC of the agent.[36] Higher concentrations of these antibiotics do not kill pathogens more rapidly. Therefore, the goal of the dosing regimen of time-dependent drugs is to optimize the duration of drug exposure. For concentration-dependent agents, such as aminoglycosides and fluoroquinolones, higher drug concentrations (i.e., above the MIC) may

result in more rapid and extensive bacterial eradication, assuming that the pathogen is sensitive to a given antibiotic. The goal of the dosing regimen with these antibiotics is to maximize drug concentration in the ocular tissues.[8,9] Note that the concentration of the antibiotic in an ophthalmal formulation (e.g., levofloxacin 0.5% or 1.5%) does not affect the MIC of that antibiotic for a given strain. Nevertheless, in these circumstances ophthalmic products with higher concentrations of antibiotics offer better assurance that the antibiotic can be present at or above the MIC for a longer time.

In vitro dynamic studies, animal studies, and a few human studies have been conducted to determine what values for peak:MIC and area under the concentration₂₄:MIC (or AUIC) are most predictive of clinical cures for fluoroquinolones. These values could be used to compare fluoroquinolones to determine which would be the most effective in treating infections by a specific organism.[30] These models indicate that a peak:MIC ratio of 10 or higher optimize rapid bacterial killing and prevent regrowth of resistant gram-negative bacterial populations. Nevertheless, some investigators have considered lower AUIC values for moxifloxacin, levofloxacin, and gatifloxacin sufficient to prevent the development of antibiotic resistance (i.e., greater than 30[11,36,44] or greater than 87[12,32]). Animal studies have confirmed the findings of these *in vitro* pharmacodynamic models. In these studies, the AUIC showed the best linear correlation with efficacy.[3]

Because fluoroquinolones demonstrate concentration-dependent killing, a dosing frequency of only once or twice daily will attain a high peak:MIC, whereas administering doses at higher concentrations will produce a high AUIC. A fluoroquinolone regimen of doses given at higher concentrations and infrequent intervals is the most efficacious in terms of bacterial killing, eradication time, and reducing the selection of resistant bacteria. An AUIC value of 75 or above for fluoroquinolones, like moxifloxacin, indicates that these agents would be effective in eradicating specific organisms. In contrast, the favorable pharmacokinetic profiles, particularly the high area under the concentration of ciprofloxacin, ofloxacin, and levofloxacin, are not sufficient to overcome their high MICs.[6,7,25,50]

In addition, peak:MIC and AUIC usually measure serum concentrations of drugs, not drug concentrations at the site of infection. Therapeutic efficacy depends on the concentration of antibiotics at the target site, primarily the concentration of free, or unbound, drug. Most fluoroquinolones rapidly penetrate ocular tissues, achieving tissue concentrations that are generally higher than those found in plasma. Using plasma concentrations as a guide frequently

underestimates the potential for clinical efficacy in ophthamology.[21,30]

Therapeutic Index

The therapeutic index, or inhibitory quotient, is another pharmacodynamic model used to evaluate antibiotic potency. It is based on drug concentration at the ultimate site of action, in tissues rather than in serum, and this makes this methodology relevant to ophthalmology. Higher tissue concentration is a significant factor in determining antibiotic efficacy and preventing resistance.[21] The therapeutic index combines *in vitro* MIC data with *in vivo* penetration data to compare the clinical efficacy of antibiotics (F5). The therapeutic index is calculated by dividing the concentration of a drug (e.g., average peak level) that is achievable in the target tissue by the drug's MIC for that pathogen. C_{max} is used to calculate the therapeutic index because the bacterial rate of killing is a function of antibiotic concentration.[27] Therefore, the ratio of C_{max} to MIC and the therapeutic index are the same. An adequate therapeutic index is 1 or higher. Higher values for the therapeutic index indicate greater drug potency or efficacy (F5, F6, F8).

Ocular Penetration of Fourth-Generation Fluoroquinolones

Moxifloxacin can be expected to achieve higher concentrations in the tear film and greater penetration into anterior ocular tissues than older fluoroquinolones because it is more soluble at the normal physiologic pH of 7.0 at the ocular surface and is both lipophilic and hydrophilic. Moxifloxacin is formulated at a pH of 6.8, and gatifloxacin at a pH of 6.0. In addition, moxifloxacin is available in a higher concentration than gatifloxacin (0.5% vs 0.3%). In combination, moxifloxacin achieves higher tissue penetration than gatifloxacin. Published data (concentrations achieved by moxifloxacin and gatifloxacin in intact rabbit corneas) suggest real differences in the ocular penetration of these fluoroquinolones (F7, F8).[35,39] A solution of moxifloxacin at 0.3% (i.e., 40%

lower or 0.2% less than the commercial formulation) achieved high concentrations in rabbit ocular tissues within 30 minutes after a single dose: 12.5 μg/mL in the cornea and 1.8 μg/mL in the aqueous humor. In contrast, gatifloxacin 0.3% achieved concentrations of 4.5 μg/mL in the cornea and 0.27 μg/mL in the aqueous humor at 1 hour after administration of a single dose. Moxifloxacin reached almost a three-fold higher concentration in the cornea compared with gatifloxacin in approximately half the time.

A recent open-label human pharmacokinetic study (F9) measured moxifloxacin penetration into the aqueous humor in human adults undergoing cataract surgery. The C_{max} for patients who received topical moxifloxacin preoperatively exceeded the MIC values for *S. aureus* and *Staphylococcus epidermidis*, suggesting that moxifloxacin may be an effective prophylactic antibiotic for endophthalmitis. These findings confirmed the rabbit data (F8), which demonstrated that levels up to 1.84 μg/mL of moxifloxacin could be achieved in the anterior chamber after topical administration. Using the human penetration data and the MIC data, the therapeutic index for moxifloxacin against *S. aureus* can be calculated. The C_{max} in the aqueous humor is 1.84 μg/mL divided by 0.06 μg/mL, the MIC for *S. aureus*. Therefore, the therapeutic index for moxifloxacin against *S. aureus* is 30.7, which indicates that topically applied moxifloxacin ophthalmic solution 0.5% achieves therapeutic concentrations in the aqueous humor, delivering 30 times the minimum amount needed to inhibit growth of organisms, like *S. aureus*. Metzler et al recently reported an MIC_{90} value of 0.063 μg/mL and an MPC_{90} value of 0.25 μg/mL against clinical isolates of methicillin-susceptible *S. aureus*.[25] Therapeutic indexes for moxifloxacin using these values would be 29.2 and 7.36, respectively. As such, not only does moxifloxacin deliver substantially more drug than is necessary to inhibit bacterial growth, but it also delivers substantially more drug than required to prevent the selection of resistance by the MPC model.

Limitations of the Therapeutic Index

Moxifloxacin and gatifloxacin penetrate the anterior chamber better than the older-generation agents. This in turn suggests that the therapeutic

F5 McGreal JA: Therapeutic drugs: are you using the best weapon against bacterial keratitis? Rev Optometry Online 1999. Available at: http://www.revoptom.com/index.asp) viewed June 14, 2005.

F6 Sheppard JD: A new generation to treat infection. Rev Ophthalmol Online 2003. Available at: http://www.revophth.com/index.asp) viewed June 14, 2005.

F7 Batoosingh AL, Lee E, Welty DF, Tang-Liu D: Gatifloxacin 0.3% vs. ciprofloxacin 0.3%: ocular pharmacokinetic profile following topical application in rabbits (abstract). Invest Ophthalmol Vis Sci 44(Suppl):2117, 2003.

F8 Robertson SM, Sanders M, Jasheway D, et al: Penetration and distribution of moxifloxacin and ofloxacin into ocular tissues and plasma following topical ocular administration in pigmented rabbits (abstract). Invest Ophthalmol Vis Sci 44(Suppl):1454, 2003.

F9 Katz HR, Masket S, Lane SS, et al: Human aqueous humor penetration pharmacokinetics of moxifloxacin after topical administration of moxifloxacin 0.5% ophthalmic solution. Abstract 10, presented at meeting of Ocular Microbiology & Immunology Group, 2003.

indexes of moxifloxacin and gatifloxacin is higher than those of older agents. More clinical studies are needed to correlate the therapeutic index with clinical outcomes for specific ocular infections. Because the therapeutic index is calculated using the drug concentration at the site of infection, it may be a better description of antibiotic potency and a more reliable method for comparing antibiotics. However, a drug's therapeutic index does not account for variability in tissue levels, any synergy or antagonism among concomitant drugs, or the time it takes for a drug to reach peak concentration (F5).

Mutant Prevention Concentrations

Blondeau et al[7] used MPCs to compare antibiotic potencies of five fluoroquinolones against *Streptococcus pneumoniae*. There was a hierarchy of potency based on MPCs and an antibiotic's ability to prevent the growth of first-step mutants. Moxifloxacin had the lowest MPC and therefore the best activity in this investigation.

Summary

Studies of endophthalmitis and keratitis isolates have shown that fourth-generation fluoroquinolones have MICs against gram-positive organisms lower than those against second- and third-generation agents. Therefore, they have greater *in vitro* potency against these ocular pathogens. Potency against gram-positive organisms is especially important because the majority of ocular infections, particularly postoperative endophthalmitis and keratitis, are caused by gram-positive organisms.[14,33]

Because pharmacodynamic models, primarily peak:MIC and AUIC, consider the *in situ* concentrations of antibiotics, they could provide a more accurate prediction of the clinical efficacy of fluoroquinolones than MIC values alone.[41] However, clinical studies have not been conducted using these models to specifically evaluate the potency of fourth-generation fluoroquinolones against gram-positive ocular infections and to compare those values with clinical outcomes. Because the therapeutic index is calculated using the drug concentration at the site of infection as well as the MIC, it is the most reliable predictor of the clinical efficacy among fluoroquinolones used to treat ocular infections. Studies in animals and humans correlated the therapeutic index and other measures of fluoroquinolone potency with clinical outcomes in specific ocular infections. These studies have demonstrated excellent achievable concentrations of moxifloxacin and gatifloxacin in the cornea and aqueous humor (F7, F8).[35] These findings, taken in conjunction with the lower MICs

found for both agents, indicate that the newer fluoroquinolones may ultimately improve clinical outcomes for patients with serious ophthalmic infections, including endophthalmitis and keratitis (F10).

Method of Literature Search

We performed an international literature search for this article based on MEDLINE database searches from 1990 to 2005, using varying combinations of the search terms *ocular infections, ophthalmic antibiotics, fluoroquinolones, moxifloxacin, potency, therapy, prophylaxis,* and *future.* All relevant journal articles and/or abstracts were selected for review. English abstracts were used for non-English papers.

References

1. Abshire R, Cockrum P, Crider J, et al: Topical antibacterial therapy for mycobacterial keratitis: potential for surgical prophylaxis and treatment. Clin Ther 26:191–6, 2004
2. Aliprandis E, Ciralsky J, Lai H, et al: Comparative efficacy of topical moxifloxacin versus ciprofloxacin and vancomycin in the treatment of *P. aeruginosa* and ciprofloxacin-resistant MRSA keratitis in rabbits. Cornea 24:201–5, 2005
3. Andes DR, Craig WA: Pharmacodynamics of fluoroquinolones in experimental models of endocarditis. Clin Infect Dis 27:47–50, 1998
4. Andrews JM: Determination of minimum inhibitory concentrations. J Antimicrob Chemother 48(Suppl 1):S5–S16, 2001
5. Blondeau JM: Fluoroquinolones: mechanism of action, classification, and development of resistance. Surv Ophthalmol 49(Suppl 2):S75–78, 2004
6. Blondeau JM, Hansen G, Metzler K, Hedlin P: The role of PK/PD parameters to avoid selection and increase of resistance: mutant prevention concentration. J Chemother 16(Suppl 3):S1–19, 2004
7. Blondeau JM, Zhao X, Hansen G, et al: Mutant prevention concentrations of fluoroquinolones for clinical isolates of *Streptococcus pneumoniae*. Antimicrob Agents Chemother 45:433–8, 2001
8. Craig WA: Choosing an antibiotic on the basis of pharmacodynamics. Ear Nose Throat J 77(Suppl):S7–12, 1998
9. Craig WA: Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin Infect Dis 12:1–18, 1998
10. Dalhoff A: Pharmacodynamics of fluoroquinolones. J Antimicrob Chemother 43(Suppl B):S51–9, 1999
11. Dalhoff A, Schmitz FJ: In vitro antibacterial activity and pharmacodynamics of new quinolones. Eur J Clin Microbiol Infect Dis 22:203–21, 2003
12. Drusano GL, Preston SL, Fowler C, et al: Relationship between fluoroquinolone area under the curve: minimum inhibitory concentration ratio and the probability of eradication of the infecting pathogen, in patients with nosocomial pneumonia. J Infect Dis 189:1590–7, 2004
13. Gunderson BW, Ross GH, Ibrahim KH, et al: What do we really know about antibiotic pharmacodynamics? Pharmacotherapy 21:302S–18S, 2001

F10 Robertson SM, Sanders M, Jasheway D, Trawick D, Curry S, Dahlin DC: Absorption and distribution of moxifloxacin, ofloxacin and gatifloxacin into ocular tissues and plasma following topical ocular administration to pigmented rabbits. Invest Ophthalmol Vis Sci E. Abstract 45: 4906, 2004.

14. Han DP, Wisniewski SR, Wilson LA, et al: Spectrum and susceptibilities of microbiologic isolates in the Endophthalmitis Vitrectomy Study. Am J Ophthalmol 122:1–17, 1996

15. Hwang DG: Fluoroquinolone resistance in ophthalmology and the potential role for newer ophthalmic fluoroquinolones. Surv Ophthalmol 49(Suppl 2):S79–83, 2004

16. Jacobs MR, Bajaksouzian S, Zilles A, et al: Susceptibilities of *Streptococcus pneumoniae* and *Haemophilus influenzae* to 10 oral antimicrobial agents based on pharmacodynamic parameters: 1997 U.S. surveillance study. Antimicrob Agents Chemother 43:1901–8, 1999

17. Kaatz GW, Moudgal VV, Seo SM: Identification and characterization of a novel efflux-related multidrug resistance phenotype in *Staphylococcus aureus*. J Antimicrob Chemother 50:833–8, 2002

18. Kahlmeter G, Brown DF, Goldstein FW, et al: European harmonization of MIC breakpoints for antimicrobial susceptibility testing of bacteria. J Antimicrob Chemother 52:145–8, 2003

19. Kishii R, Takei M, Fukuda H, et al: Contribution of the 8-methoxy group to the activity of gatifloxacin against type II topoisomerases of *Streptococcus pneumoniae*. Antimicrob Agents Chemother 47:77–81, 2003

20. Kowalski RP, Dhaliwal DK, Karenchak LM, et al: Gatifloxacin and moxifloxacin: an *in vitro* susceptibility comparison to levofloxacin, ciprofloxacin, and ofloxacin using bacterial keratitis isolates. Am J Ophthalmol 136:500–5, 2003

21. Liu P, Müller M, Derendorf H: Rational dosing of antibiotics: the use of plasma concentrations versus tissue concentrations. Int J Antimicrob Agents 19:285–90, 2002

22. MacGowan AP, Wise R: Establishing MIC breakpoints and the interpretation of *in vitro* susceptibility tests. J Antimicrob Chemother 48(Suppl 1):S17–28, 2001

23. Mah FS: Fourth-generation fluoroquinolones: new topical agents in the war on ocular bacterial infections. Curr Opin Ophthalmol 15:316–20, 2004

24. Mather R, Karenchak LM, Romanowski EG, et al: Fourth generation fluoroquinolones: new weapons in the arsenal of ophthalmic antibiotics. Am J Ophthalmol 133:463–6, 2002

25. Metzler K, Hansen GM, Hedlin P, et al: Comparison of minimal inhibitory and mutant prevention drug concentrations of 4 fluoroquinolones against clinical isolates of methicillin-susceptible and -resistant *Staphylococcus aureus*. Int J Antimicrob Agents 24:161–7, 2004

26. NCCLS: National Committee for Clinical Laboratory Standards: Performance Standards for Antimicrobial Susceptibility Testing; Twelfth Informational Supplement. Document M100–S12. Wayne, PA, NCCLS, 2002

27. Neu HC, Elmer PD: The inhibitory quotient. Bull N Y Acad Med 59:430–42, 1983

28. Olson RJ: Reducing the risk of postoperative endophthalmitis. Surv Ophthalmol 49(Suppl 2):S55–61, 2004

29. Pestova E, Millichap JJ, Noskin GA, et al: Intracellular targets of moxifloxacin: a comparison with other fluoroquinolones. J Antimicrob Chemother 45:583–90, 2000

30. Pickerill KE, Paladino JA, Schentag JJ: Comparison of the fluoroquinolones based on pharmacokinetic and pharmacodynamic parameters. Pharmacotherapy 20:417–28, 2000

31. Piddock LJV, Jin YF: Antimicrobial activity and accumulation of moxifloxacin in quinolone-susceptible bacteria. J Antimicrob Chemother 43(Suppl B):S39–42, 1999

32. Preston SL, Drusano GL, Berman AL, et al: Pharmacodynamics of levofloxacin: a new paradigm for early clinical trials. JAMA 279:125–9, 1998

33. Pushker N, Dada T, Sony P, et al: Microbial keratitis after laser in situ keratomileusis. J Refract Surg 18:280–6, 2002

34. Rhee MK, Kowalski RP, Romanowski EG, et al: A laboratory evaluation of antibiotic therapy for ciprofloxacin-resistant *Pseudomonas aeruginosa*. Am J Ophthalmol 138:226–30, 2004

35. Robertson SM, Curtis MA, Schlech BA, et al: Ocular pharmacokinetics of moxifloxacin after topical treatment of animals and humans. Surv Opthalmol 50(Suppl 1):S32–S45, 2005

36. Schentag JJ, Gilliland KK, Paladino JA: What have we learned from pharmacokinetic and pharmacodynamic theories? Clin Infect Dis 32(Suppl 1):S39–46, 2001

37. Siefert HM, Domdey-Gette A, Henninger K, et al: Pharmacokinetics of the 8-methoxyquinolone, moxifloxacin: a comparison in humans and other mammalian species. J Antimicrob Chemother 43(Suppl B):S69–76, 1999

38. Siefert HM, Kohlsdorfer C, Steinke W, et al: Pharmacokinetics of the 8-methoxyquinolone, moxifloxacin: tissue distribution in male rats. J Antimicrob Chemother 43(Suppl B):61–7, 1999

39. Solomon R, Donnenfeld ED, Perry HD, et al: Penetration of topically applied gatifloxacin 0.3%, moxifloxacin 0.5%, and ciprofloxacin 0.3% into the aqueous humor. Opthalmology 112(3):466–9, 2005

40. Soussy CJ, Nguyen J, Goldstein F, et al: In vitro antibacterial activity of moxifloxacin against hospital isolates: a multicentre study. Clin Microbiol Infect 9:997–1005, 2003

41. Tipperman R: Pharmacologic considerations for cataract surgery. Curr Opin Ophthalmol 15:51–5, 2004

42. Wagner RS, Abelson MB, Shapiro A, Torkildsen G: Evaluation of moxifloxacin, ciprofloxacin, gatifloxacin, ofloxacin and levofloxacin concentrations in human conjunctival tissue. Arch Opthalmol 123:1282–5, 2005

43. Wright DH, Brown GH, Peterson ML, et al: Application of fluoroquinolone pharmacodynamics. J Antimicrob Chemother 46:669–83, 2000

44. Zhanel GG, Walters M, Laing N, et al: In vitro pharmacodynamic modelling simulating free serum concentrations of fluoroquinolones against multidrug-resistant *Streptococcus pneumoniae*. J Antimicrob Chemother 47:435–40, 2001

ELSEVIER

In Vitro and In Vivo Potency of Moxifloxacin and Moxifloxacin Ophthalmic Solution 0.5%, A New Topical Fluoroquinolone

David W. Stroman, PhD, Joseph J. Dajcs, PhD, Gale A. Cupp, MS, and Barry A. Schlech, PhD

Alcon Research, Ltd., Fort Worth, TX, USA

Abstract. Fluoroquinolones are a class of synthetic antibacterial agents that were approved for ocular therapy in 1991 and have become popular therapy for the treatment and prevention of various ocular infections. These agents are synthetic, broad-spectrum, rapidly bactericidal, and have good penetration into ocular tissues. Their main mechanism of action is the inhibition of bacterial enzymes needed for bacterial DNA synthesis. However, antibiotic resistance occurred swiftly to the earlier fluoroquinolones and better fluoroquinolones were needed. The fourth-generation fluoroquinolones, such as moxifloxacin and gatifloxacin, have enhanced activity against gram-positive bacteria while retaining potent activity against most gram-negative bacteria. These fourth-generation fluoroquinolones have improved penetration into the anterior chamber and have also demonstrated increased in vivo efficacy in several animal models of ocular infections. In addition, topical ophthalmic antibiotic products can deliver antibiotic concentrations directly to the eye that are thousands of times higher than their MICs. This article reviews published data describing the in vitro potency of moxifloxacin and its in vivo activity for treating and preventing experimental ocular infections. (Surv Ophthalmol 50:S16–S31, 2005. © 2005 Elsevier Inc. All rights reserved.)

Key words. antibiotic activity • antibiotic potency • antibiotic resistance • in vitro • in vivo • MICs • moxifloxacin • VIGAMOX®

Introduction

Fluoroquinolones are synthetic, broad-spectrum, bactericidal antibiotics that were approved for treatment of ocular infections in 1991. The effectiveness of second- and third-generation fluoroquinolones (e.g., ofloxacin, ciprofloxacin, levofloxacin) has been offset by the emergence of fluoroquinolone-resistant organisms.[46,68,73] The fourth-generation fluoroquinolones (moxifloxacin and gatifloxacin) show an enhanced spectrum of activity against gram-positive

bacteria and comparable activity to second- and third-generation fluoroquinolones (ciprofloxacin and levofloxacin) against gram-negative bacteria.[7,11,12,25,26] New ocular formulations with improved potency, such as moxifloxacin ophthalmic solution 0.5% (VIGAMOX®, Alcon Laboratories, Fort Worth, TX) or gatifloxacin ophthalmic solution 0.3% (Zymar®, Allergan, Irvine, CA), are currently available and have been shown to inhibit growth of organisms resistant to second- and third-generation

S16

fluoroquinolones.[78] The purpose of this article is to review a) the *in vitro* activity of moxifloxacin against clinical ocular isolates and b) the *in vivo* effectiveness of moxifloxacin ophthalmic solution 0.5% in treating or preventing experimental ocular infections.

Mechanism of Action

The fluoroquinolones are potent antibacterial agents that target bacterial enzymes necessary for DNA synthesis (i.e., replication, transcription, repair, and recombination). These important bacterial enzymes are DNA gyrase and topoisomerase IV.[10,31-35] The principal event in the action of the fluoroquinolone is the trapping of gyrase or topoisomerase IV on DNA as ternary drug-enzyme-DNA complexes.[31,34,116] The fluoroquinolone-enzyme-DNA complexes prevent uncoiling and/or separation of the replicated strands of DNA, resulting in the inhibition of DNA replication and death of the bacterium.[40,52] The breaks in the double-stranded DNA result in the death of the replicating cell.[32,83,116]

Individual fluoroquinolones target either DNA gyrase or topoisomerase IV and in some cases, both. The DNA gyrase is the target in organisms, such as *Mycobacterium tuberculosis, Helicobacter pylori,* and *Treponema pallidum,* which represent a group of bacteria with genomes that lack a gene encoding topoisomerase IV.[33] In gram-negative organisms DNA gyrase is more sensitive than topoisomerase IV to fluoroquinolones and is considered to be the primary target.[9,33,36,37,40,85,126] The target of fluoroquinolones for gram-positive organisms is more complex and varies based on the individual fluoroquinolone, as demonstrated in Table 1 for *Staphylococcus aureus* and

TABLE 1

Inhibitory Activities of Fluoroquinolones Against DNA Gyrase and Topoisomerase IV

	IC$_{50}$ (µg/mL)		
Fluoroquinolone	DNA Gyrase	Topoisomerase IV	IC$_{50}$ Ratio[b]
From *Staphylococcus aureus*[a]			
Ciprofloxacin	13.5	5.76	0.43
Ofloxacin	18.8	22.8	1.21
Levofloxacin	8.06	9.81	1.22
Moxifloxacin	3.44	7.84	2.28
From *Streptococcus pneumoniae*[c]			
Moxifloxacin	8.02 (20 µM)	4.01 (10 µM)	0.5
Gatifloxacin	7.5–15.0	3.6–7.5	0.5
	(20–40 µM)	(10–20 µM)	
Levofloxacin	28.9 (80 µM)	14.4 (40 µM)	0.5
Ciprofloxacin	13.25 (40 µM)	7.6 (20 µM)	0.5

[a] From Takei M et al.[109]
[b] IC$_{50}$ Ratio: IC$_{50}$ against topoisomerase IV/IC$_{50}$ against DNA gyrase.
[c] Calculated from Yague G et al.[122]

Streptococcus pneumoniae. The gyrase in these organisms is less sensitive to fluoroquinolones than in gram-negative bacteria.[32] The general target in gram-positive organisms is topoisomerase IV.[9,36,37,40,85,109,122] This enzyme tends to have about the same sensitivity in both organisms. The fourth-generation fluoroquinolones target both DNA gyrase and topoisomerase IV.[10,33,38,42,101]

Mechanisms of Resistance

Resistance to fluoroquinolones emerged shortly after the introduction of the second-generation compounds, ofloxacin and ciprofloxacin.[3,46,73] Resistance to fluoroquinolones requires significant genetic changes in one or more of four major bacterial mechanisms: a) enzymes for DNA synthesis, b) gyrase protecting proteins, c) cell permeability, or d) drug efflux.[56,99] Also, enzymes that degrade fluoroquinolones have not been reported in bacteria, but have been found in fungi.[119] Fluoroquinolone resistance develops in a step-wise fashion. Lowered susceptibility has been associated with porins that regulate intracellular drug concentration or changes in proteins that protect target enzymes from attack. These changes may occur spontaneously and the mutants are subsequently selected by suboptimal fluoroquinolone therapy.[93] Much of the resistance has been caused by the systemic, agricultural, and vetinary use of fluoroquinolones and less as a result of topical administration. Wegener and Engberg provided an excellent review of the past veterinary use of quinolones, and discourage such use to preserve the efficacy of quinolones for human use.[118] Ciprofloxacin has been licensed for use in swine and chickens in Asia and Latin America. Ofloxacin has been licensed for use in chickens and turkeys in Japan and Asia. Topical application of fluoroquinolones tends to produce less resistance because the concentration of drug introduced to the site of infection is several hundred, and often several thousand times, the MIC against common organisms. The ability of topical antibiotic products for the eye, such as moxifloxacin ophthalmic solution 0.5%, to deliver 5,000 µg/ml of antibiotic directly onto the infected tissue is formidable. The 5,000 µg/ml is 10,000 times the MIC of 0.5 µg/ml, a common MIC for this antibiotic against ocular isolates.

The following discussion reviews each of four major mechanisms by which organisms become resistant to a particular antibiotic.

CHANGES IN ENZYME TARGETS

Point mutations in the genes encoding DNA gyrase or topoisomerase IV reduce the affinity of the fluoroquinolones to these enzymes.[90] DNA gyrase is a complex of GyrA and GyrB subunits encoded by the *gyrA*

and *gyrB* genes that introduce supercoiling into DNA in a reaction driven by the hydrolysis of ATP. DNA gyrase is essential for initiation of DNA replication and has a role in elongation by removing positive supercoils from DNA as a result of unwinding at the replication fork. Topoisomerase IV is also involved in DNA replication by the decatenation of linked daughter chromosome during the end stages of replication. Topoisomerase IV is composed of two subunits ParC and ParE that are encoded by the *parC* and *parE* genes, respectively.

Resistance in gram-negative bacteria occurs typically as a result of alterations in DNA gyrase, either in the GyrA or GyrB subunit.[126] Mutations in the GyrA subunit have a tendency to cluster in the quinolone resistance-determining region (QRDR). QRDR represents the region of the *gyrA* gene, which encodes the GyrA subunit that binds to DNA during enzyme activation.[126] Mutations in QRDR are thought to cause resistance through decreased drug affinity for the altered gyrase-DNA complex.[120] Mutations to the GyrB subunit occur less frequently than mutations to the GyrA subunit. Whether GyrB mutations affect fluoroquinolone binding remains unclear;[125] however, alterations in GyrB subunit produce lower levels of resistance as compared to GyrA subunit mutations.[55]

Fluoroquinolone resistance in gram-positive organisms generally result from mutations to topoisomerase IV subunits by alterations to the ParC or ParE subunits, with mutations in ParC playing a more prominent role in resistance.[39,85] Many fluoroquinolones primarily target topoisomerase IV of *S. aureus*, but the target in *S. pneumoniae* varies among the fluoroquinolones.[87,88,109,122] Furthermore, the fourth-generation fluoroquinolones were reported to target both DNA gyrase and topoisomerase IV.[10,33,38,42,87,89,101] Topoisomerase IV mutations have been studied in gram-negative bacteria and are believed to play a secondary role in development of resistance as ParC or ParE mutations typically confer resistance only in the presence of concomitant DNA gyrase mutations.[17,63]

Genetic studies have shown that DNA gyrase is the primary target of quinolones in *Escherichia coli*. In genetic studies, single mutations in either GyrA or GyrB subunits of DNA gyrase conferred first-step and subsequent incremental drug resistance.[51] Additional studies demonstrated that topoisomerase IV is a secondary drug target in *E. coli*.[63] Genetic studies with *S. aureus* showed that first-step drug resistance was found in the mutants with point mutations to the ParC or ParE subunits of topoisomerase IV, indicating topoisomerase IV is the primary target of quinolones.[39,85,109] The genetic data are not as straightforward for other species, however, there

does appear to be a pattern that shows DNA gyrase is the primary quinolone target in gram-negative bacteria and that topoisomerase IV is the primary drug target in gram-positive bacteria.[56]

PRODUCTION OF GYRASE PROTECTION PROTEIN

All of the genetic loci and mutations that confer resistance to fluoroquinolones are chromosomally mediated with the exception of a plasmid-mediated gyrase-protecting protein recently described in *Klebsiella pneumoniae*.[76,95] The plasmid found primarily in gram-negative organisms contains the *qnr* (quinolone resistance) locus that confers resistance by encoding a 218-amino acid protein that protects DNA gyrase from the fluoroquinolones.[113] The frequency of clinical isolates containing the *qnr* determinant is unknown.[95]

DECREASING CELL PERMEABILITY

In order for fluoroquinolones to access their targets in the cytoplasm, they must traverse the cell wall and cytoplasmic membrane of all bacteria and the outer membrane of gram-negative organisms. The cell wall of gram-positive organisms is believed not to be a barrier to diffusion of fluoroquinolones and other small molecules of 300–400 Da. Porins in the outer membrane are responsible for regulating drug diffusion. However, in gram-negative organisms, decreased levels of porins in the outer membrance reduce the accumulation of fluoroquinolone in the cytoplasm.[53,92]

EFFECTIVE EFFLUX PUMPS

The bacteria's efflux pump mechanism contributes to bacterial resistance by preventing lethal levels of fluoroquinolone from accumulating in the cytoplasm.[55] The efflux pump is a mechanism that expels the fluoroquinolone across the cell membrane and out of the cell, thereby reducing the intracellular concentration to sublethal levels.[27,55] The action of the efflux pump is dependent on the ability of fluoroquinolones to bind to the bacterial efflux protein which expells it from the cell. Some fluoroquinolones, particularly moxifloxacin, are less affected by bacterial efflux mechanisms due to their bulky side-chain moiety at position 7 that hinders its export out of the cell.[91]

CONCLUSION

Mutations that confer resistance to second- and third-generation quinolones also lower susceptibility to fourth-generations quinolones. An isolate with reduced susceptibility to one fluoroquinolone will be less susceptible to all fluoroquinolones; however,

the reduced susceptibility may or may not be above the breakpoint definition of resistance. Gram-positive bacteria are less likely to be resistant to the fourth-generation fluoroquinolones due to their enhanced potency. The second- and third-generation fluoroquinolones can select for mutations in either DNA gyrase or topoisomerase IV and produce a situation where bacteria will need only one additional mutation to become resistant to the fourth-generation fluoroquinolones. The preferential use of the fourth-generation fluoroquinolones for gram-positive ocular infections may delay the emergence of resistant isolates. Bacteria are less likely to develop resistance to the fourth-generation fluoroquinolones than the second-generation due to the dual targeting.

In Vitro Potency

IN VITRO SUSCEPTIBILITY OF BACTERIA RECOVERED FROM OCULAR INFECTIONS

Moxifloxacin has been shown to possess potent *in vitro* activity against a wide spectrum of bacteria and is more active against *Staphylococcus* and *Streptococcus* species than previous generation fluoroquinolones.[7,41,45,54] Table 2 (gram-positives), 3 (gram-negatives), and 4 (atypicals) present a comparison of intrinsic susceptibilities to fluoroquinolones for bacterial species routinely encountered in ocular infections. Intrinsic susceptibility to an antibiotic is typified by the median (50%) minimal inhibitory concentration of the organisms tested (MIC_{50}), unless more than 50% of the isolates of the specific species have acquired resistance to the antibiotic. Intrinsic susceptibility to a specific antibiotic for a particular species is the same regardless from where the isolates are recovered (i.e., geographical areas or sites of infection). This allows comparisons of fluoroquinolone susceptibilities of bacteria from different sites of infection.

Several studies have compared the activity of moxifloxacin and other fluoroquinolones against clinical bacterial isolates from ocular and nonocular sites.[7,12,41,45,54,67,108] Table 2, 3, and 4 show comparative *in vitro* data, which generally demonstrate that moxifloxacin is more active than the earlier generation fluoroquinolones (ciprofloxacin, ofloxacin, and levofloxacin), especially against staphylococci, streptococci, and a variety of atypical organisms.[100] Based on their reported MICs against atypical mycobacteria, like *Mycobacterium chelonae*, *M. kansasii*, and *M. fortuitum*, moxifloxacin and gatifloxacin exhibit more favorable *in vitro* susceptibility results than do ciprofloxacin, levofloxacin, and ofloxacin (Table 4).[1,2,44,96,100] Moxifloxacin has been shown to be slightly more active than gatifloxacin against staphylococci and streptococci.[7,41]

FREQUENCY AND SIGNIFICANCE OF RESISTANT ISOLATES IN OCULAR INFECTIONS

After the introduction of ciprofloxacin into medical practice in 1985–1987, reports of ciprofloxacin resistance in *Staphylococcus* appeared almost immediately (within a year).[14,98] The emergence of ciprofloxacin resistance, especially in MRSA strains, in hospitals caused concern for the future usefulness of fluoroquinolones for treating *S. aureus* infections.[28,58,66,74,82,103] Apparently, the tremendous amounts of fluoroquinolones used around the world and its inappropriate use in the past two decades has diminished the initial effectiveness due to the rapid emergence of ciprofloxacin-resistant strains. By the time ciprofloxacin ophthalmic solution 0.3% (Ciloxan®, Alcon Laboratories, Fort Worth, TX) was introduced to the American market in 1991, the fluoroquinolone resistance rate in *S. aureus* isolates from systemic infections was already 11%[64] and 14% in ocular infections.[3] Furthermore, retrospective studies of clinical keratitis isolates found the resistance rate of *S. aureus* had increased to 28–35% by 1998.[3,46] Ciprofloxacin resistance was reported for gram-positive organisms; however, resistance is less common in gram-negative organisms.[43,64,110]

There are two primary approaches to define antibiotic resistance. The first approach is the breakpoint MIC method. The National Committee for Clinical Laboratory Standards (NCCLS) has defined MIC breakpoints for many antibiotics used to treat infections, especially blood borne, urinary tract, and respiratory tract infections (F1, F2). Breakpoint susceptibility testing uses designated antibiotic concentrations necessary to differentiate between the interpretive categories of "susceptible," "intermediate," or "resistant," rather than a range of five or more doubling-dilution concentrations used to determine MICs.[60] A specific breakpoint MIC is defined for each antibiotic, above which if the isolate survives and grows it is considered to be resistant to that antibiotic. The MIC breakpoint usually is predictive of clinical efficacy for systemic therapy because it is set with the antibiotic's specific pharmacokinetic parameters in view. Resistant isolates would likely fail systemic therapy. The second approach is the acquired resistance approach. This approach recognizes the intrinsic susceptibility of a particular species

---

[F1] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; M100-S15, Fifteenth Informational Supplement. Wayne PA: Jan 2005.

[F2] National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Susceptibility Testing; Twelfth Informational Supplement. Wayne, PA: NCCLS, 2002. Document M100-S12, 2002.

TABLE 2

Susceptibility of Gram-Positive Species to Five Fluoroquinolones

	MIC$_{50}$ (µg/ml)				
Bacterial Species	Moxifloxacin	Gatifloxacin	Levofloxacin	Ciprofloxacin	Ofloxacin
Staphylococci					
Staphylococcus aureus	0.03[a,b,c]	0.06[a], 0.094[b]	0.13[a], 0.19[b]	0.25[a,c], 0.38[b]	0.50[b,c]
Staphylococcus epidermidis	0.06[a,b,c,d]	0.13[a,b,d]	0.19[b,d], 0.50[a]	0.22[b,d], 0.25[c], 0.50[a]	.50[b,c,d]
Staphylococcus haemolyticus	0.06[a,c]	0.13[a]	0.50[a]	0.50[a,c]	0.50[c]
Staphylococcus saprophyticus	0.03[a], 0.13[c]	0.06[a]	0.25[a]	0.25[a], 0.50[c]	1.0[c]
Staphylococcus lugdunensis	0.13[c]	-	-	0.25[c]	0.50[c]
Staphylococcus hominis	0.03[a], 0.06[c]	0.13[a]	0.50[a]	0.13[c], 0.50[a]	0.25[c]
Staphylococcus simulans	0.03[a]	0.06[a]	0.25[a]	0.25[a]	-
Staphylococcus pasteuri	0.06[c]			0.25[c]	0.50[c]
Staphylococcus warneri	0.03[a], 0.06[c]	0.13[a]	0.25[a]	0.25[a,c]	0.50[c]
Streptococci and Enterococci					
Streptococcus pneumoniae	0.06[c], 0.13[a,b]	0.22[b], 0.25[a]	0.75[c], 1.0[a]	0.50[c], 0.75[b], 1.0[a]	1.0[c], 2.0[b]
Streptococcus mitis	0.13[c]			2.0[c]	2.0[b,c]
Streptococcus, viridans group	0.19[b,c]	0.25[b]	0.75[b]	1.0[b], 2.0[c]	2.0[b,c]
Streptococcus pyogenes	0.13[c], 0.25[a]	0.25[a]	1.0[a]	0.50[c], 1.0[a]	1.0[c]
Enterococcus faecalis	0.19[b,d], 0.25[a,c]	0.38[b,d], 0.50[a]	0.75[b,d], 1.0[a]	0.75[b,d], 1.0[a,c]	2.0[b,d,c]
Micrococci					
Micrococcus luteus	0.50[c]	-	-	1.0[c]	2.0[c]
Kocuria spp.	0.25[c]			1.0[c]	2.0[c]
Bacilli					
Bacillus cereus	0.09[b,e], 0.13[c]	0.09[b,e]	0.13[b,e]	0.13[b,c,e]	0.25[c], 0.38[b,e]
Bacillus pumilus	0.13[c]	-	-	0.13[c]	0.13[c]
Bacillus subtilis	0.016[c]			0.13[c]	0.13[c]
Coryneforms					
Corynebacterium accolens	0.03[c]	-	-	0.06[c]	0.25[c]
Corynebacterium macginleyi	0.06[c]			0.03[c]	0.13[c]
Corynebacterium propinquium	0.25[c]			0.25[c]	1.0[c]
Corynebacterium pseudodiphtheriticum	0.25[c]			0.25[c]	1.0[c]

[a] Published data from Bauernfeind et al.[7]
[b] Published data from Kowalski et al[67] and Mather et al.[78]
[c] Unpublished data from Alcon.
[d] Coagulase-negative *Staphylococcus* rather than *Staphylococcus epidermidis.*
[e] *Enterococcus* spp. rather than *Enterococcus faecalis.*
[f] *Bacillus* spp. rather than *Bacillus cereus.*

to an antibiotic and considers any isolate with a 4- to 16-fold increase in MIC above the intrinsic susceptibility to have acquired resistance. There is no implied relationship between isolates defined as resistant by this method and clinical outcome. This method is appropriate when attempting to define genetic changes that confer resistant phenotypes.

Two terms, "MIC$_{90}$" and "% resistant" are useful in expressing the prevalence of resistant isolates within a group of isolates of a particular species to a specific antibiotic. If less than 10% of the isolates are resistant, the MIC$_{90}$ will be similar to the MIC$_{50}$. If 10–49% of the isolates within the group are resistant, the MIC$_{90}$ will be higher than the MIC$_{50}$. If more than 50% of the isolates are resistant, the MIC$_{50}$ will be higher as well. Therefore, the number or frequency of isolates of a particular species that are resistant cannot be derived from the MIC$_{90}$, but rather must be calculated directly as a percentage of the total number of isolates of the specific species tested.

The frequency of encountering fluoroquinolone-resistant isolates prior to therapy has increased during the last 15 years in several important species recovered from healthy ocular surfaces as well as from ocular infection.[3,15,19,46,57,59,71,75,114,117] Most of these reports define resistance as any isolate with a ciprofloxacin MIC of 2.0 µg/ml or greater. The resistance breakpoint for ofloxacin is 1.0 µg/ml. The current NCCLS resistant breakpoints (F1) for moxifloxacin are established for *Staphylococcus* species as greater than or equal to 2.0 µg/ml and for *Streptococcus pneumoniae* as greater than or equal to 4.0 µg/ml. There are no resistant breakpoints for moxifloxacin for any other bacterial species. There is still debate about their relevance.[106]

Significant geographical differences in the frequencies of resistant isolates have been reported in ocular infections.[3,19,46,67,75,97] Particularly important is the rapid emergence of fluoroquinolone- and methicillin-resistant *Staphylococcus aureus* (MRSA) in

TABLE 3

Susceptibility of Gram-Negative Species to Five Fluoroquinolones

Bacterial Species	MIC$_{90}$ (μg/ml)				
	Moxifloxacin	Gatifloxacin	Levofloxacin	Ciprofloxacin	Ofloxacin
Enterobacericeae					
Aeromonas caviae	0.13[c]	-	-	0.03[c]	0.06[c]
Citrobacter koseri	0.03[c]			0.008[c]	0.06[c]
Enterobacter aerogenes	0.06[a], 0.25[c]	0.06[a]	0.06[a]	0.03[a], 0.13[c]	0.25[c]
Enterobacter cloacae	0.03[a], 0.13[c]	0.016[a]	0.03[a]	0.016[a], 0.13[c]	0.25[c]
Enterobacter hormaechei	0.13[c]			0.03[c]	0.13[c]
Escherichia coli	0.008[a], 0.06[c]	0.008[a]	0.016[a]	0.008[a], 0.03[c]	0.13[c]
Klebsiella oxytoca	0.03[a], 0.25[c]	0.016[a]	0.03[a]	0.016[a], 0.03[c]	0.13[c]
Klebsiella pneumoniae	0.03[a], 0.13[c]	0.03[a]	0.03[a]	0.016[a], 0.06[c]	0.25[c]
Morganella morganii	0.06[a], 0.50[c]	0.06[a]	0.03[a]	0.016[a], 0.06[c]	0.50[c]
Pantoea agglomerans	0.03[a], 0.06[c]	0.016[a]	0.03[a]	0.016[a], 0.03[c]	0.13[c]
Proteus mirabilis	0.06[a], 0.50[c]	0.13[a]	0.03[a]	0.016[a], 0.03[c]	0.25[c]
Serratia marcescens	0.25[a,b], 0.50[c]	0.19[b], 0.25[a,b]	0.03[a]	0.064[b], 0.13[a,c]	0.50[b,c]
Nonfermentative					
Achromobacter xylosoxidans	2.0[c], 4.0[a]	8.0[a]	8.0[a]	2.0[c], 4.0[a]	2.0[c]
Acinetobacter baumannii	0.03[a], 0.13[c]	0.06[a]	0.06[a]	0.13[a], 0.25[c]	0.25[c]
Acinetobacter calcoaceticus	0.016[a], 0.06[c]	0.016[a]	0.06[a]	0.13[a], 0.25[c]	0.25[c]
Acinetobacter johnsonii	0.016[a], 0.13[c]	0.016[a]	0.06[a]	0.06[a], 0.25[c]	0.50[c]
Acinetobacter junii	0.06[c]			0.25[c]	0.25[c]
Acinetobacter genospecies 3	0.016[a], 0.06[c]	0.016[a]	0.06[a]	0.13[a], 0.25[c]	0.25[c]
Chryseobacterium indologenes	0.25[c]			1.0[c]	1.0[c]
Chryseomonas luteola	0.13[c]			0.03[c]	0.13[c]
Stenotrophomonas maltophilia	0.13[a], 1.0[c]	0.25[a]	0.25[a]	0.50[a], 4.0[c]	4.0[c]
Pseudomonads					
Pseudomonas aeruginosa	0.50[c], 2.0[c], 4.0[a]	0.25[b], 4.0[a]	0.38[b], 2.0[a]	0.094[b], 0.25[c], 0.50[a]	0.75[b], 2.0[c]
Pseudomonas orzyihabitans	0.13[c]			0.03[c]	0.13[c]
Pseudomonas stutzeri	0.25[a,c]	0.13[a]	0.13[a]	0.03[a,c]	0.13[c]
Others					
Haemophilus influenzae	0.016[a], 0.03[c], 0.039[b,d]	0.008[a], 0.017[b,d]	0.024[b,d], 0.03[a]	0.008[a,c], 0.014[b,d]	0.03[c], 0.05[b,d]
Moraxella catarrhalis	0.03[a], 0.047[b,e], 0.06[c]	0.03[a,b,e]	0.016[a], 0.047[b,e]	0.016[a], 0.032[b,c,e]	0.13[b,c,e]
Moraxella osloensis	0.13[c]			0.13[c]	0.25[c]
Neisseria perflava	0.03[c]			0.008[c]	0.03[c]

[a] Published data from Bauernfeind et al.[7]
[b] Published data from Kowalski et al.[67]
[c] Unpublished data from Alcon.
[d] *Haemophilus* spp. rather than *Haemophilus influenzae*.
[e] *Moraxella* spp. rather than *Moraxella catarrhalis*.

hospital settings. With the recent market introduction of the fourth-generation fluoroquinolones (moxifloxacin and gatifloxacin) it is important to note that isolates resistant to second- and third-generation fluoroquinolones have decreased susceptibility to fourth-generation fluoroquinolones as well (Table 5). Ciprofloxacin-resistant isolates can be divided into two groups, those with moderate levels of resistance (2 to 8 μg/ml) and those with high-level resistance (16 μg/ml or higher). Very few isolates with moderate levels of resistance to ciprofloxacin were, in fact, resistant to fourth-generation fluoroquinolones. However, isolates that had a high-level resistance to ciprofloxacin were also classified as resistant to moxifloxacin. Table 6 presents recent data for ocular *S. aureus* and *S. epidermidis* isolates recovered from conjunctivitis from three different parts of the world. Based on the MIC$_{90}$s for moxifloxacin and ciprofloxacin, the 979 strains of *Staphylococcus aureus* and *S. epidermidis* are clearly more susceptible to moxifloxacin than ciprofloxacin throughout the three regions. Seppala et al reported *in vitro* resistance to moxifloxacin in up to 2.2% of *Streptococcus viridans* isolates from the normal flora of patients prior to cataract surgery.[102]

In spite of the increase in the number of fluoroquinolone-resistant pathogens recovered from ocular infections, there have not been reports of a corresponding increase in the number of treatment failures. In fact, it was recognized by NCCLS that its resistance breakpoint definition has no predictive value of topical therapy success or failure (F2).

TABLE 4

Susceptibility of Selected Atypical and Anaerobic Species to Five Fluoroquinolones

Bacterial Species	MIC$_{50}$ (µg/ml)				
	Moxifloxacin	Gatifloxacin	Levofloxacin	Ciprofloxacin	Ofloxacin
Atypicals					
Mycobacterium avium	3.2[a]	6.4[a]	-	-	-
Mycobacterium marinum	0.4[a]	0.4[a]			
Mycobacterium chelonae	1.6[a], 8.0[b]	1.6[a], 8.0[b]	32[b]	16[b]	-
Mycobacterium abcessus	8.0[b]	8.0[b]	16[b]		
Mycobacterium fortuitum group	0.06[b]	0.12[b]	0.25[b]	0.25[b]	-
Mycobacterium kansasii	0.06[d]		0.12[d]		
Chlamydia trachomatis	0.03[c]	-	0.25[c]	1.0[c]	1.0[c]
Anaerobes					
Propionibacterium acnes	0.25[c]	-	0.50[c]	0.50[c]	0.50[c]
Bacteroides fragilis	0.25[c]	-	1.0[c]	4.0[c]	2.0[c]
Clostridium perfringens	0.50[c]	-	0.50[c]	1.0[c]	1.0[c]
Peptostreptococcus spp.	0.25[c]	-	0.50[c]	0.50[c]	1.0[c]

[a] Published data from Saito et al.[96]
[b] Published data from Yang et al.[124]
[c] Published data from Fung-Tomc et al.[41]
[d] Published data from Alcaide et al.[2]

TABLE 5

Comparative Quinolone-Resistance in Ocular Isolates of Staphylococcus species[a]

Bacterial Species	Resistance Level	n	Moxifloxacin			Ciprofloxacin		
			MIC Range	MIC$_{50}$	MIC$_{90}$	MIC Range	MIC$_{50}$	MIC$_{90}$
S. aureus	Moderate	20	0.016–2.0	0.13	2.0	2.0–8.0	2.0	8.0
	High	50	2.0–>32	4.0	8.0	16–≥128	128	≥128
S. epidermidis	Moderate	83	0.06–1.0	1.0	1.0	2.0–8.0	4.0	8.0
	High	48	0.50–>32	2.0	32	16–128	64	64
S. haemolyticus	Moderate	26	0.13–1.0	1.0	1.0	2.0–8.0	2.0	4.0
	High	13	1.0–8.0	2.0	4.0	16–≥128	16	≥128

[a] Unpublished data from Alcon.

TABLE 6

Geographic Differences in Fluoroquinolone Susceptibility in Staphylococcus Isolates Recovered from Conjunctivitis

Bacterial Species[a]	Region	n	Moxifloxacin			Ciprofloxacin		
			MIC Range	MIC$_{50}$	MIC$_{90}$	MIC Range	MIC$_{50}$	MIC$_{90}$
S. aureus[b]	USA	96	0.016–8.0	0.03	4.0	0.13–>128	0.25	64
	Europe	146	0.016–4.0	0.06	0.13	0.13–>128	0.25	1.0
	India	59	0.016–2.0	0.03	1.0	0.13–32	0.25	2.0
S. epidermidis	USA	335	0.03–>32	0.06	0.25	0.06–>128	0.25	1.0
	Europe	216	0.03–32	0.06	0.13	0.06–64	0.25	0.50
	India	127	0.03–2.0	0.06	0.50	0.13–64	0.25	4.0
	Total	979						

[a] Unpublished data from Alcon (**F3**).
[b] Includes MRSA and MSSA.

Topically administered moxifloxacin ophthalmic solution 0.5% delivers high concentrations (i.e.,

5,000 µg/ml) directly to the bacteria in superficial ocular infections. This concentration is 1,000 to 2,000 times that needed to inhibit growth of isolates classified as resistant *in vitro*. If the site of the ocular infection is deeper into the eye, the concentration of any topical antibiotic penetrating beyond the corneal

surface and into intraocular sites over time becomes critical. It may, in fact, be insufficient to inhibit the growth of a resistant organism in the posterior aspects of the eye.

MUTANT PREVENTION CONCENTRATION

A major concern with the use of any antibacterial agent is as to what extent the use of the agent causes resistant strains to emerge. Although MICs give information about a specific antibiotic concentration that inhibits the growth of a bacterial strain, it does not distinguish between the bactericidal or bacteriostatic effects. A new term has been recently defined for fluoroquinolones is called mutant prevention concentration (MPC).[13,29]

Drlica reviewed this concept and proposed a "mutant selection window" which is an antimicrobial concentration range extending from the minimal inhibitory concentration required to block the growth of wild-type bacteria up to that required to inhibit the growth of the least susceptible, single-step mutant.[30] The upper boundary is the MPC. The MPC is often equivalent to the MIC of the most resistant mutant of a heterogeneous bacterial population. In general, the MPC will be 8 to 10-fold higher than the MIC for a reasonably susceptible isolate. An important technical difference between the two approaches is that MIC testing challenges the antibiotic with 10^5 colony forming units (CFUs), whereas MPC testing generally uses 10^{10} CFUs. The range of concentrations between MIC and MPC is defined as the mutant selection window.[32,127] Considerable efforts are being made to define the relationship between pharmacodynamic properties of the antibacterial agent and the mutant-selection window.[5,21] The length of time to maintain the antibacterial agent concentration above the MPC at the site of infection and its impact on emergence of resistant strains has yet to be clinically established. The MPC is an important concept in preventing the growth of organisms that have some level of drug resistance prior to therapy. The concentration of antibiotic must be maintained above the MPC to resist the selection of mutants that would give rise to a population of organisms that are not susceptible to the antibiotic.

KINETICS OF KILL TESTING

Kinetics of kill studies are *in vitro* tests that assess the rate of killing by a particular antibiotic of specific bacterial isolates. For technical reasons, testing is almost always performed at a constant concentration of antibiotic throughout the entire time of exposure of drug to the logarithmically growing bacteria. This *in vitro* assay may be useful in distinguishing the

bacteriostatic and bactericidal effects of many antibiotics. Kinetics of kill studies have been performed with antibiotic concentrations equal to multiples of the MIC; for example, 2X MIC, 4X MIC, 8X MIC, 10X MIC, and so forth. Moxifloxacin is rapidly bactericidal in such studies.[16] Another testing approach uses dilutions of antibiotic products. For example, in superficial infections such as conjunctivitis, testing has been performed at 1:10 and 1:100 dilutions of the product to represent the tear film concentrations at 10 and 30 minutes after topical dosing.

Staphylococci represent the most frequently isolated group of bacteria from superficial ocular infections (e.g., conjunctivitis and blepharitis), deeper ocular infections (e.g., endophthalmitis), as well as from the conjunctiva and skin/lid lash margins of healthy eyes. The kinetics of kill by moxifloxacin of *S. aureus* and *S. epidermidis* (quinolone-susceptible and quinolone-resistant) isolates, was recently reported (F3). As shown in Figs. 1 and 2, moxifloxacin, at 500 μg/ml and 50 μg/ml, kills faster than ciprofloxacin at 300 and 30 μg/ml. These concentrations were tested as they correspond to 1:10 and 1:100 dilutions of the formulated products. Nevertheless, the faster kill by moxifloxacin compared to ciprofloxacin makes it an important option when rapid eradication of organisms on or near the surface of the eye is warranted. Rapid eradication minimizes the amplification of subpopulations of resistant strains.[121,127]

In Vivo Potency

ASSESSMENT OF FLUOROQUINOLONE THERAPY IN RABBIT KERATITIS MODELS

Several reports have established the effectiveness of fluoroquinolones in the treatment of experimental keratitis. Data from Barequet et al have shown the improved efficacy of a third-generation fluoroquinolone (trovafloxacin) as compared to ciprofloxacin and ofloxacin in a rabbit model of *S. aureus* and *P. aeruginosa* keratitis.[6] Their results showed that the third-generation fluoroquinolone had approximately a 2-log greater reduction in CFU/cornea relative to the second-generation fluoroquinolone in treating *S. aureus* and all the fluoroquinolones studied sterilized the corneas in their *P. aeruginosa* model.[6]

Furthermore, other studies using a rabbit model of keratitis induced by corneal injection of *P. aeruginosa* demonstrated that there were no significant differences between moxifloxacin and ciprofloxacin in the treatment of *P. aeruginosa.*[4]

The *in vivo* efficacies of commercial solutions of 0.5% moxifloxacin (VIGAMOX®, Alcon, Fort Worth,
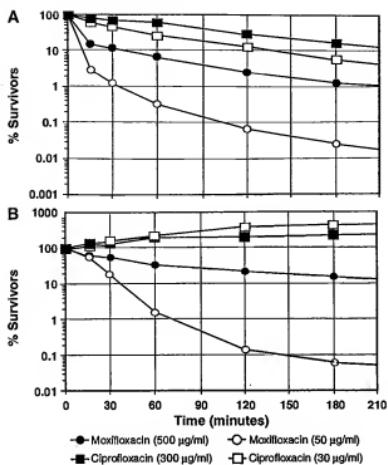
Fig. 1. Kinetics of kill of *Staphylococcus aureus*. A: Quinolone-sensitive (ciprofloxacin MIC = 0.25 μg/ml) *S. aureus* were incubated with moxifloxacin or ciprofloxacin and the percent survivors were determined at various time points. B: Quinolone-resistant (ciprofloxacin MIC = 128 μg/ml) *S. aureus* were incubated with moxifloxacin or ciprofloxacin and the percent survivors were determined at various time points.
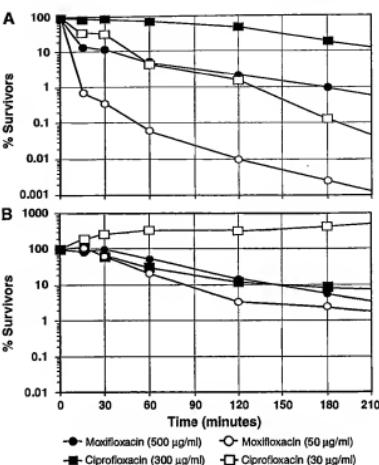
Fig. 2. Kinetics of kill of *Staphylococcus epidermidis*. A: Quinolone-sensitive (ciprofloxacin MIC = 0.13 μg/ml) *S. epidermidis* were incubated with moxifloxacin or ciprofloxacin and the percent survivors were determined at various time points. B: Quinolone-resistant (ciprofloxacin MIC = 64 μg/ml) *S. epidermidis* were incubated with moxifloxacin or ciprofloxacin and the percent survivors were determined at various time points.

TX), 0.3% levofloxacin (Quixin®), and 0.3% ciprofloxacin (Ciloxan®) were compared in the treatment of experimental keratitis caused by *S. aureus* (with various sensitivities to methicillin and second-generation fluoroquinolones),[24] *P. aeruginosa*, and *Serratia marcescens* keratitis in rabbits.[112] Two treatment models were used with the *S. aureus*: an early treatment model where bacteria are actively replicating and a late treatment model where bacteria were in their stationary phase of growth. *P. aeruginosa* and *S. marcescens* keratitis models involved treatment of bacteria in their slow replication phase, but not yet in their stationary phase. The results from these animal studies of ocular infections treated with fluoroquinolones are shown in Figs. 3–6 and reviewed below.

TREATMENT OF FLUOROQUINOLONE-SENSITIVE *STAPHYLOCOCCUS AUREUS* KERATITIS

Early treatment of rabbit eyes infected with ofloxacin-sensitive MSSA or MRSA demonstrated that moxifloxacin, levofloxacin, or ciprofloxacin reduced the number of *S. aureus* equally by approximately 5-log CFU/cornea as compared to the untreated control group (Figs. 3A and 3B).[24] Eyes with established infections caused by bacteria that are not actively replicating are more refractory to antibiotics. Therefore, moxifloxacin, levofloxacin, and ciprofloxacin were evaluated to determine their effectiveness in treating such infections. Late treatment of infected rabbit eyes with moxifloxacin, levofloxacin, or ciprofloxacin produced approximately 5, 4, or 2.5-log reduction in CFU/cornea, respectively, relative to the control group (Figs. 3C and 3D).[24] None of the fluoroquinolones tested were as effective as moxifloxacin in reducing *S. aureus* in the late treatment model. Moxifloxacin was shown to be the most effective therapy demonstrating its activity in both the early and later treatment schedules. *S. aureus* produce tissue destructive exoproteins as the growth of the organisms goes from an actively replicating state (log phase) to a stationary phase of growth. During the stationary phase is where inflammation occurs and is the point when patients seek medical attention.
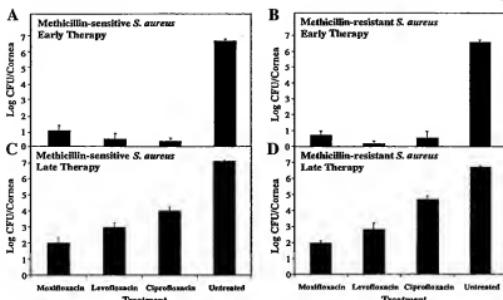
Fig. 3. Early and late fluoroquinolone treatment of ofloxacin-sensitive *Staphylococcus aureus* keratitis in rabbits. Early (*A* and *B*) and late (*C* and *D*) fluoroquinolone therapy of rabbit eyes infected with MSSA or MRSA sensitive to ofloxacin (MIC = 0.5 µg/ml). (Reprinted from Dajcs et al[24] with permission of the American Society for Microbiology.)

TREATMENT OF FLUOROQUINOLONE-RESISTANT *STAPHYLOCOCCUS AUREUS* KERATITIS

Early treatment of rabbit eyes infected with ofloxacin-resistant MSSA or MRSA with moxifloxacin, levofloxacin, or ciprofloxacin produced approximately 4.5, 3.5, or 0.5-log reductions in CFU/cornea, respectively, relative to the untreated eyes (Figs. 4A and 4B).[24]

Late treatment of the infected rabbit eyes with either levofloxacin or ciprofloxacin did not produce significant reductions in CFU relative to the untreated control (Figs. 4C and 4D).[24] During late treatment, only moxifloxacin was able to significantly

reduce the CFU/cornea as compared to the untreated group.

TREATMENT OF *PSEUDOMONAS AERUGINOSA* KERATITIS

A study by Rhee et al employed a rabbit keratitis model to demonstrate that ciprofloxacin-sensitive *P. aeruginosa* (MIC = 2) can be effectively treated with ciprofloxacin.[94] Additionally, they showed that ciprofloxacin-resistant *P. aeruginosa* (MIC > 32) was not effectively treated with ciprofloxacin.[93] Treatment of *P. aeruginosa* keratitis with moxifloxacin, levofloxacin, ciprofloxacin, or ofloxacin resulted in a 5
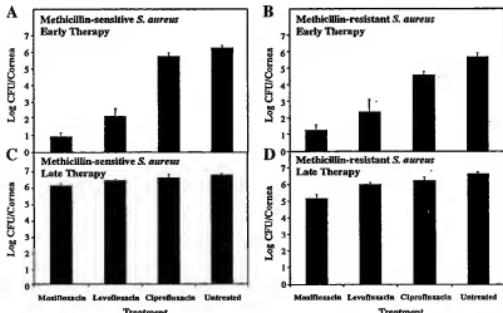
Fig. 4. Early and late fluoroquinolone treatment of ofloxacin-resistant *Staphylococcus aureus* keratitis in rabbits. Early (*A* and *B*) and late (*C* and *D*) fluoroquinolone therapy of rabbit eyes infected with MSSA or MRSA resistant to ofloxacin (MIC = 128 µg/ml). (Reprinted from Dajcs et al[24] with permission of the American Society for Microbiology.)
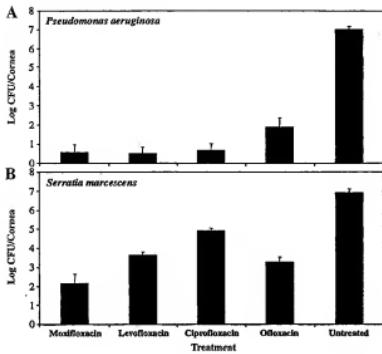
Fig. 5. Fluoroquinolone treatment of P. aeruginosa or S. marcescens keratitis in rabbits. A: Fluoroquinolone therapy of rabbit eyes infected with ciprofloxacin-sensitive P. aeruginosa (MIC = 0.13 µg/ml). B: Fluoroquinolone therapy of rabbit eyes infected with ciprofloxacin-sensitive S. marcescens (MIC = 0.13 µg/ml). (Figure adapted from Thibodeaux BA et al.[112])

or greater log reduction in CFU/cornea as compared to the untreated group (Fig. 5).[112] These data demonstrate that moxifloxacin is equal to or more effective in treating infections caused by P. aeruginosa then the second- and third-generation fluoroquinolones like ciprofloxacin or levofloxacin.
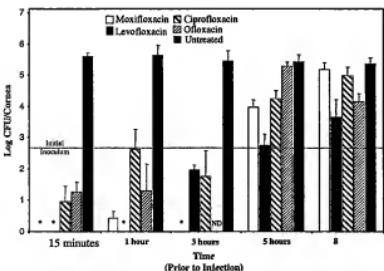
Fig. 6. Fluoroquinolone prophylaxis of experimental keratitis caused by Staphylococcus aureus. Rabbit eyes were treated with a single topical application of fluoroquinolone at various time points prior to inoculation with approximately 500 CFU of S. aureus (ciprofloxacin MIC = 0.5 µg/ml). * - Sterile eyes; ND - not determined. (Based on data from Dajcs et al.[22,23])

TREATMENT OF SERRATIA MARCESCENS KERATITIS

Treatment of S. marcescens keratitis with moxifloxacin, levofloxacin, ciprofloxacin, or ofloxacin produced approximately a 5, 3, 2, 3.5-log reduction in CFU/cornea, respectively, relative to the untreated control (Fig. 5).[112] Moxifloxacin was the most effective therapy in the reduction in CFUs of S. marcescens in the cornea.

PROPHYLAXIS OF EXPERIMENTAL STAPHYLOCOCCUS KERATITIS

Recent reports have shown that the occurrence of postsurgical infections following ocular surgery is increasing.[20,61,84] Although other organisms, like Streptococcus can cause endophthalmitis,[80] the most common organisms isolated in postsurgical endophthalmitis are S. epidermidis and S. aureus[48,105] and are derived mainly from the patient's own eyelids.[47,105] Given the ability of surface microbial flora to enter the eye during surgery, prophylactic antibiotics have been administered before, during and after ocular surgery to reduce the risk of postsurgical infections, especially keratitis or endophthalmitis.[8,70]

In order to prevent postoperative ocular infections, a drug must reach the relevant tissues at appropriate concentrations. Topical ophthalmic preparation must overcome tear dilution, mechanical action of the eyelids, and rapid run off over the concave corneal surface. Antimicrobial agents with good ocular penetration in the tear film, cornea, and aqueous humor are needed to prevent post-surgical ocular infections.[86] Topical antibiotics may reduce the risk of post-surgical infection by sterilizing the ocular surface, preventing the introduction of bacteria into the eye by maintaining a lethal concentration in ocular tissue, thereby killing any exogenous bacteria introduced during surgery.

Ophthalmologists have been using fluoroquinolones for prophylactic treatment to reduce the risk of ocular infections for many years. Fluoroquinolones have been used prophylactically prior to ocular surgery because of their broad-spectrum of coverage and good penetration into tissues and aqueous humor.[77,79] Ciprofloxacin and ofloxacin[18,49,123] and levofloxacin[65] have been shown to achieve significant intraocular concentrations. Furthermore, fourth-generation fluoroquinolones, such as moxifloxacin and gatifloxacin, have been shown to achieve higher concentrations in the aqueous humor than older fluoroquinolones.[50,72,79] (F4) Levine et al demonstrated that the topical application of moxifloxacin produced approximately a 45% higher concentration in the aqueous humor when comparison to gatifloxacin.[72] Hariprasad et al found that topical administration of moxifloxacin ophthalmic solution

0.5% every 2 or 6 hours for 3 days before surgery resulted in antibiotic concentrations of 2.28 µg/ml in the aqueous humor and 0.11 µg/ml in the vitreous in 2-hour group and 0.88 µg/ml (aqueous) and 0.06 µg/ml (vitreous) in the 6-hour group.[50] There is no consensus as to how frequently an antibiotic should be administered for adequate prophylaxis. In Miller's review of eye infections, corneal concentrations achievable by topical administration of fluoroquinolones ranged from 0.04 to 5.29 µg/ml.[81] Robertson et al reported ocular penetration of moxifloxacin up to 24.8 µg/g cornea, which was five times greater than that for gatifloxacin (4.85 µg/ml) after topical administration in rabbits.[22] Other investigators saw a two-fold increase of moxifloxacin over gatifloxacin in the aqueous humor of humans (F4).[F4] A recent study compared timing and frequency of topical ofloxacin administered 1 hour or 3 days prior to surgery.[107] Additional studies have indicated a reduction in positive conjunctival cultures both immediately before and after surgery, signifying the importance of prophylactic topical antibiotic therapy.[86,107]

Several studies have evaluated second-generation fluoroquinolones (i.e., ciprofloxacin and ofloxacin) as prophylactic antibiotics for the prevention of experimental S. aureus keratitis.[22,23,115] Another study showed the effectiveness of a fourth-generation fluoroquinolone (gatifloxacin) in the prevention of multi-drug-resistant S. aureus keratitis after lamellar keratectomy in a rabbit model.[115] The fluoroquinolones were quantitatively evaluated for their effectiveness in preventing growth of the S. aureus inoculum by applying fluoroquinolone at various times prior to inoculation of bacteria and enumerating viable cell counts.

Data presented in Fig. 6 show a comparison of prophylactic effectiveness of moxifloxacin, levofloxacin, and two second-generation fluoroquinolones. All fluoroquinolones when applied 3 hours or less prior to inoculation prevented an increase in the number of bacteria relative to the initial inoculum (500 CFU); however, only moxifloxacin sterilized all the eyes. None of the fluoroquinolones were effective in reducing the CFU relative to the inoculum when applied at 5 or 8 hours prior to infection. In this prophylaxis model of keratitis, topical moxifloxacin demonstrated superior penetration across an intact corneal epithelium and to a lethal concentration, for up to 3 hours in the cornea and anterior chamber at concentrations that were lethal to the organisms at the time of injection.

Topical moxifloxacin applied pre-challenge, post-challenge, or pre-and post-challenge has been demonstrated to prevent endophthalmitis with an inoculum of 50,000 CFU of S. aureus into the anterior chamber.[69] This is the first report demonstrating that topical application of antibiotic can prevent experimental endophthalmitis.

Tungsiripat et al have also demonstrated the improved effectiveness of a fourth-generation fluoroquinolones in relation to second and third-generation fluoroquinolones for the prevention of multiple-drug-resistant S. aureus keratitis after lamellar keratectomy.[115] Their data showed that ciprofloxacin or levofloxacin were 45% effective in preventing keratitis, whereas, gatifloxacin was 100% effective in preventing multiple drug-resistant S. aureus keratitis. These experiments have established that fourth-generation fluoroquinolones can be successful prophylactic antibiotic for the prevention of keratitis or endophthalmitis. According to Thauvin-Eliopoulos, it is both the broad-spectrum antimicrobial activity of these drugs, and their high potency against many susceptible strains, that position the fluoroquinolone antimicrobials among our most valuable therapeutic classes.[111]

Conclusions

In vitro studies have shown that moxifloxacin has improved activity for gram-positive and atypical organisms and similar activity against gram-negative organisms compared to second and third-generation fluoroquinolones (i.e., ofloxacin, ciprofloxacin, levofloxacin). Moxifloxacin inhibited the growth of bacteria frequently isolated from ocular infections and had a faster rate of killing of fluoroquinolone-resistant organisms than ciprofloxacin. Furthermore, in vivo studies demonstrated that moxifloxacin ophthalmic solution 0.5% is an effective topical therapy for treatment or prevention of experimental bacterial keratitis. Moxifloxacin penetrates anterior ocular tissues (e.g., cornea, tear film, conjunctiva, iris-ciliary body, aqueous humor) to concentrations at or above the MIC of the major ocular pathogens.[22,62] The high intraocular concentrations achieved coupled with the inate in vitro and in vivo potency, as well as the inhibition of the bacterial efflux mechanism enhances the ability of moxifloxacin to accumulate to lethal levels at the infected site and thereby minimize the rate of resistance to the drug.

Method of Literature Search

A literature search for this article was performed based on MEDLINE database searches from 1966 to 2005, using varying combinations of the search terms

---

[F4] McCulley JP, Surratt G, Shine W: 4th generation fluoroquinolone penetration into aqueous humor in humans. Invest Ophthalmol Vis Sci 45: Abstract 4927, 2004.

ocular infections, fluoroquinolones, generations, mechanism of action, mutant prevention concentration, ocular penetration, prophylaxis, animal models of keratitis, keratitis, endophthalmitis, and resistance. Relevant journal articles were selected for review. Articles cited in the references of journal articles were also included. An effort to use the most recently available literature was made, concentrating on journal articles published in the last decade.

References

1. Abshire R, Cockrum P, Crider J, et al: Topical antibacterial therapy for mycobacterial keratitis: potential for surgical prophylaxis and treatment. Clin Ther 26:191–6, 2004
2. Alcaide F, Calatayud L, Santin M, et al: Comparative *in vitro* activities of linezolid, telithromycin, clarithromycin, levofloxacin, moxifloxacin, and four conventional antimycobacterial drugs against *Mycobacterium kansasii*. Antimicrob Agents Chemother 48:4562–5, 2004
3. Alexandrakis G, Alfonso EC, Miller D: Shifting trends in bacterial keratitis in south Florida and emerging resistance to fluoroquinolones. Ophthalmology 107:1497–502, 2000
4. Aliprandis E, Ciralsky J, Lai H, et al: Comparative efficacy of topical moxifloxacin versus ciprofloxacin and vancomycin in the treatment of *P. aeruginosa* and ciprofloxacin-resistant MRSA keratitis in rabbits. Cornea 24:201–5, 2005
5. Allen GP: The mutant prevention concentration (MPC): a review. J Infect Dis Phamacother 6:27–47, 2003
6. Barequet IS, Denton P, Osterhout GJ, et al: Treatment of experimental bacterial keratitis with topical trovafloxacin. Arch Ophthalmol 122:65–9, 2004
7. Bauernfeind A: Comparison of the antibacterial activities of the quinolones Bay 12-8039, gatifloxacin (AM 1155), trovafloxacin, clinafloxacin, levofloxacin and ciprofloxacin. J Antimicrob Chemother 40:639–51, 1997
8. Beigi B, Westlake W, Chang B, et al: The effect of intracameral, pre-operative antibiotics on microbial contamination of anterior chamber aspirates during phacoemulsification. Eye 12(Pt 3a):390–4, 1998
9. Bianche F, Cameron B, Bernard FX, et al: Differential behaviors of *Staphylococcus aureus* and *Escherichia coli* type II DNA topoisomerases. Antimicrob Agents Chemother 40:2714–20, 1996
10. Blondeau JM: Fluoroquinolones: mechanism of action, classification, and development of resistance. Surv Ophthalmol 49(Suppl 2):S73–S78, 2004
11. Blondeau JM: A review of the comparative in-vitro activities of 12 antimicrobial agents, with a focus on five new respiratory quinolones. J Antimicrob Chemother 43(Suppl B):1–11, 1999
12. Blondeau JM, Laskowski K, Bjarnason J, et al: Comparative *in vitro* activity of gatifloxacin, grepafloxacin, levofloxacin, moxifloxacin and trovafloxacin against 4151 Gram-negative and Gram-positive organisms. Int J Antimicrob Agents 14:45–50, 2000
13. Blondeau JM, Zhao X, Hansen G, Drlica K: Mutant prevention concentrations of fluoroquinolones for clinical isolates of *Streptococcus pneumoniae*. Antimicrob Agents Chemother 45:433–8, 2001
14. Blumberg HM, Rimland D, Carroll DJ, et al: Rapid development of ciprofloxacin resistance in methicillin-susceptible and -resistant *Staphylococcus aureus*. J Infect Dis 163:1279–85, 1991
15. Boswell FJ, Andrews JM, Jevons G, et al: Comparison of the *in vitro* activities of several new fluoroquinolones against respiratory pathogens and their abilities to select fluoroquinolone resistance. J Antimicrob Chemother 50:495–502, 2002
16. Boswell FJ, Andrews JM, Wise R, et al: Bactericidal properties of moxifloxacin and post-antibiotic effect. J Antimicrob Chemother 43(Suppl B):43–9, 1999
17. Breines DM, Ouabdesselam S, Ng EY, et al: Quinolone resistance locus nfxD of *Escherichia coli* is a mutant allele of the parE gene encoding a subunit of topoisomerase IV. Antimicrob Agents Chemother 41:175–9, 1997
18. Cekic O, Batman C, Totan Y, et al: Penetration of ofloxacin and ciprofloxacin in aqueous humor after topical administration. Ophthalmic Surg Lasers 30:465–8, 1999
19. Chaudhry NA, Flynn HW, Murray TG, et al: Emerging ciprofloxacin-resistant *Pseudomonas aeruginosa*. Am J Ophthalmol 128:509–10, 1999
20. Colleaux KM, Hamilton WK: Effect of prophylactic antibiotics and incision type on the incidence of endophthalmitis after cataract surgery. Can J Ophthalmol 35:373–8, 2000
21. Croisier D, Etienne M, Bergoin E, et al: Mutant selection window in levofloxacin and moxifloxacin treatments of experimental pneumococcal pneumonia in a rabbit model of human therapy. Antimicrob Agents Chemother 48:1699–707, 2004
22. Dajcs JJ, Moreau JM, Stroman DW, et al: The effectiveness of tobramycin and Ocuflox in a prophylaxis model of *Staphylococcus* keratitis. Curr Eye Res 23:60–3, 2001
23. Dajcs JJ, Moreau JM, Thibodeaux BA, et al: Effectiveness of ciprofloxacin and ofloxacin in a prophylaxis model of *Staphylococcus* keratitis. Cornea 20:878–80, 2001
24. Dajcs JJ, Thibodeaux BA, Marquart ME, et al: Effectiveness of ciprofloxacin, levofloxacin, or moxifloxacin for treatment of experimental *Staphylococcus aureus* keratitis. Antimicrob Agents Chemother 48:1948–52, 2004
25. Dalhoff A: Comparative *in vitro* and *in vivo* activity of the C-8 methoxy quinolone moxifloxacin and the C-8 chlorine quinolone BAY y 3118. Clin Infect Dis 32(Suppl 1):S16–S22, 2001
26. Dalhoff A, Petersen U, Endermann R: *In vitro* activity of BAY 12-8039, a new 8-methoxyquinolone. Chemotherapy 42:410–25, 1996
27. Daporta MT, Muñoz Bellido JL, Guirao GY, et al: *In vitro* activity of older and newer fluoroquinolones against efflux-mediated high-level ciprofloxacin-resistant *Streptococcus pneumoniae*. Int J Antimicrob Agents 24:185–7, 2004
28. Daum TE, Schaberg DR, Terpenning MS, et al: Increasing resistance of *Staphylococcus aureus* to ciprofloxacin. Antimicrob Agents Chemother 34:1862–3, 1990
29. Dong Y, Zhao X, Domagala J, et al: Effect of fluoroquinolone concentration on selection of resistant mutants of *Mycobacterium bovis* BCG and *Staphylococcus aureus*. Antimicrob Agents Chemother 43:1756–8, 1999
30. Drlica K: The mutant selection window and antimicrobial resistance. J Antimicrob Chemother 52:11–7, 2003
31. Drlica K: Mechanism of fluoroquinolone action. Curr Opin Microbiol 2:504–8, 1999
32. Drlica K, Hooper DC: Mechanisms of quinolone action. in Hooper DC, Ethan Rubinstein E (eds): Quinolone Antimicrobial Agents. Washington, DC, ASM Press, 2003, 3, chap 2, pp 19–40
33. Drlica K, Malik M: Fluoroquinolones: action and resistance. Curr Top Med Chem 3:249–82, 2003
34. Drlica K, Zhao X, et al: DNA topoisomerase IV as a quinolone target. Curr Opin Antiinfect Drugs 1:435–42, 1999
35. Drlica K, Zhao X: DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiol Mol Biol Rev 61:377–92, 1997
36. Ferrero L, Cameron B, Crouzet J: Analysis of gyrA and grlA mutations in stepwise-selected ciprofloxacin-resistant mutants of *Staphylococcus aureus*. Antimicrob Agents Chemother 39:1554–8, 1995
37. Ferrero L, Cameron B, Manse B, et al: Cloning and primary structure of *Staphylococcus aureus* DNA topoisomerase IV: a primary target of fluoroquinolones. Mol Microbiol 13:641–53, 1994
38. Fisher LM, Heaton VJ: Dual activity of fluoroquinolones against *Streptococcus pneumoniae*. J Antimicrob Chemother 51:463–4;author reply 464–5, 2003

39. Fournier B, Hooper DC: Mutations in topoisomerase IV and DNA gyrase of *Staphylococcus aureus*: novel pleiotropic effects on quinolone and coumarin activity. Antimicrob Agents Chemother 42:121–8, 1998

40. Fournier B, Zhao X, Lu T, et al: Selective targeting of topoisomerase IV and DNA gyrase in *Staphylococcus aureus*: different patterns of quinolone-induced inhibition of DNA synthesis. Antimicrob Agents Chemother 44:2160–5, 2000

41. Fung-Tomc JC, Minassian B, Kolek B, et al: Antibacterial spectrum of a novel des-fluoro(6) quinolone, BMS-284756. Antimicrob Agents Chemother 44:3351–6, 2000

42. García-Rodríguez JA, Gómez García AC: The microbiology of moxifloxacin. Drugs Today (Barc) 36:215–27, 2000

43. Garg P, Sharma S, Rao GN: Ciprofloxacin-resistant *Pseudomonas* keratitis. Ophthalmology 106:1319–23, 1999

44. Gillespie SH, Billington O: Activity of moxifloxacin against mycobacteria. J Antimicrob Chemother 44:393–5, 1999

45. Goldstein EJ, Citron DM, Hudspeth M, et al: *In vitro* activity of Bay 12-8039, a new 8-methoxyquinolone, compared to the activities of 11 other oral antimicrobial agents against 390 aerobic and anaerobic bacteria isolated from human and animal bite wound and clenched fist bite tissue infections in humans. Antimicrob Agents Chemother 41:1552–7, 1997

46. Goldstein MH, Kowalski RP, Gordon YJ: Emerging fluoroquinolone resistance in bacterial keratitis: a 5-year review. Ophthalmology 106:1313–8, 1999

47. Groden LR, Murphy B, Rodnite J, et al: Lid flora in blepharitis. Cornea 10:50–3, 1991

48. Han DP, Wisniewski SR, Wilson LA, et al: Spectrum and susceptibilities of microbiologic isolates in the Endophthalmitis Vitrectomy Study. Am J Ophthalmol 122:1–17, 1996

49. Hanioglu-Kargi S, Basci N, Soysal H, et al: The penetration of ofloxacin into human aqueous humor given by various routes. Eur J Ophthalmol 8:33–6, 1998

50. Hariprasad SM, Blinder KJ, Shah GK, et al: Penetration pharmacokinetics of topically administered 0.5% moxifloxacin ophthalmic solution in human aqueous and vitreous. Arch Ophthalmol 123:39–44, 2005

51. Heaton VJ, Ambler JE, Fisher LM: Potent antipneumococcal activity of gemifloxacin is associated with dual targeting of gyrase and topoisomerase IV, an *in vivo* target preference for gyrase, and enhanced stabilization of cleavable complexes *in vitro*. Antimicrob Agents Chemother 44:3112–7, 2000

52. Hiasa H, Yousef DO, Marians KJ: DNA strand cleavage is required for replication fork arrest by a frozen topoisomerase-quinolone-DNA ternary complex. J Biol Chem 271:26424–9, 1996

53. Hirai K, Aoyana H, Irikura T, et al: Differences in susceptibility to quinolones of outer membrane mutants of *Salmonella typhimurium* and *Escherichia coli*. Antimicrob Agents Chemother 29:535–8, 1985

54. Hoogkamp-Korstanje JA, Roelofs-Willemse J: Comparative *in vitro* activity of moxifloxacin against Gram-positive clinical isolates. J Antimicrob Chemother 45:31–9, 2000

55. Hooper C: Mechanisms of fluoroquinolone resistance. Drug Resist Update 2:38–55, 1999

56. Hooper DC, Rubinstein E (eds): Quinolone Antimicrobial Agents. ASM Press, Washington, DC, 2003, ed 3

57. Hwang DG: Fluoroquinolone resistance in ophthalmology and the potential role for newer ophthalmic fluoroquinolones. Surv Ophthalmol 49(Suppl 2):S79–S83, 2004

58. Isaacs RD, Kunke PJ, Cohen RL, Smith JW: Ciprofloxacin resistance in epidemic methicillin-resistant *Staphylococcus aureus*. Lancet 2:843, 1988

59. Jones ME, Staples AM, Critchley I, et al: Benchmarking the *in vitro* activities of moxifloxacin and comparator agents against recent respiratory isolates from 377 medical centers throughout the United States. Antimicrob Agents Chemother 44:2645–52, 2000

60. Jorgensen JH, Turnidge JD: Susceptibility test methods: Dilution and disk diffusion methods. In Murray PR (ed): Manual of Clinical Microbiology. 8th ed. Washington, DC, ASM Press, 2003, pp 1108–28

61. Karp CL, Tuli SS, Yoo SH, et al: Infectious keratitis after LASIK. Ophthalmology 110:503–10, 2003

62. Katz HR, Masket S, Lane SS, et al: Absorption of topical moxifloxacin ophthalmic solution into human aqueous humor. Cornea (in press)

63. Khodursky AB, Zechiedrich EL, Cozzarelli NR: Topoisomerase IV is a target of quinolones in *Escherichia coli*. Proc Natl Acad Sci USA 92:11801–5, 1995

64. Knauf HP, Silvany R, Southern PM, et al: Susceptibility of corneal and conjunctival pathogens to ciprofloxacin. Cornea 15:66–71, 1996

65. Kobayakawa S, Tochikubo T, Tsuji A: Penetration of levofloxacin into human aqueous humor. Ophthalmic Res 35:97–101, 2003

66. Kotilainen P, Nikoskelainen J, Huovinen P: Emergence of ciprofloxacin-resistant coagulase-negative staphylococcal skin flora in immunocompromised patients receiving ciprofloxacin. J Infect Dis 161:41–4, 1990

67. Kowalski RP, Dhaliwal DK, Karenchak LM, et al: Gatifloxacin and moxifloxacin: an *in vitro* susceptibility comparison to levofloxacin, ciprofloxacin, and ofloxacin using bacterial keratitis isolates. Am J Ophthalmol 136:500–5, 2003

68. Kowalski RP, Pandya AN, Karenchak LM, et al: An *in vitro* resistance study of levofloxacin, ciprofloxacin, and ofloxacin using keratitis isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Ophthalmology 108:1826–9, 2001

69. Kowalski RP, Romanowski EG, Mah FS, et al: Topical prophylaxis with moxifloxacin prevents endophthalmitis in a rabbit model. Am J Ophthalmol 138:33–7, 2004

70. Kramer A, Behrens-Baumann W: Prophylactic use of topical anti-infectives in ophthalmology. Ophthalmologica 211 (Suppl 1):68–76, 1997

71. Kurokawa N, Hayashi K, Konishi M, et al: Increasing ofloxacin resistance of bacterial flora from conjunctival sac of preoperative ophthalmic patients in Japan. Jpn J Ophthalmol 46:586–9, 2001

72. Levine JM, Noecker RJ, Lane LC, et al: Comparative penetration of moxifloxacin and gatifloxacin in rabbit aqueous humor after topical dosing. J Cataract Refract Surg 30:2177–82, 2004

73. Low DE: Quinolone resistance and its clinical relevance, in Hooper DC and Ethan Rubinstein E (eds), Quinolone Antimicrobial Agents. ASM Press, Washington, DC. 2003, ed 3, chap 23, pp 355–86

74. Maple P, Hamilton-Miller J, Brumfitt W: Ciprofloxacin resistance in methicillin- and gentamicin-resistant *Staphylococcus aureus*. Eur J Clin Microbiol Infect Dis 8:622–4, 1989

75. Marangon FB, Miller D, Muallem MS, et al: Ciprofloxacin and levofloxacin resistance among methicillin-sensitive *Staphylococcus aureus* isolates from keratitis and conjunctivitis. Am J Ophthalmol 137:453–8, 2004

76. Martínez-Martínez L, Pascual A, Jacoby GA: Quinolone resistance from a transferable plasmid. Lancet 351:797–9, 1998

77. Masket S: Preventing, diagnosing, and treating endophthalmitis. J Cataract Refract Surg 24:725–6, 1998

78. Mather R, Karenchak LM, Romanowski EG, et al: Fourth generation fluoroquinolones: new weapons in the arsenal of ophthalmic antibiotics. Am J Ophthalmol 133:463–6, 2002

79. Mather R, Stewart JM, Prabriputaloong T, et al: The effect of cataract surgery on ocular levels of topical moxifloxacin. Am J Ophthalmol 138:554–9, 2004

80. Miller JJ, Scott IU, Flynn HW, et al: Endophthalmitis caused by *Streptococcus pneumonia*. Am J Ophthalmol 138:231–6, 2004

81. Miller MH, Mayers M: Treatment of eye infections, in Hooper DC and Ethan Rubinstein E (eds), Quinolone Antimicrobial Agents. Washington, DC, ASM Press, 2003, 3, chap 18, pp 291–309

82. Milne LM, Faiers MC: Ciprofloxacin resistance in epidemic methicillin-resistant *Staphylococcus aureus*. Lancet 2:843, 1988

83. Mizuuchi K, Fisher LM, O'Dea MH, et al: DNA gyrase action involves the introduction of transient double-strand breaks into DNA. Proc Natl Acad Sci USA 77:1847–51, 1980

84. Nagaki Y, Hayasaka S, Kadoi C, et al: Bacterial endophthalmitis after small-incision cataract surgery. effect of incision placement and intraocular lens type. J Cataract Refract Surg 29:20–6, 2003

85. Ng EY, Trucksis M, Hooper DC: Quinolone resistance mutations in topoisomerase IV: relationship to the flqA locus and genetic evidence that topoisomerase IV is the primary target and DNA gyrase is the secondary target of fluoroquinolones in *Staphylococcus aureus*. Antimicrob Agents Chemother 40:1881–8, 1996

86. Olson RJ: Reducing the risk of postoperative endophthalmitis. Surv Ophthalmol 49(Suppl 2):S55–S61, 2004

87. Pan XS, Fisher LM: *Streptococcus pneumoniae* DNA gyrase and topoisomerase IV: overexpression, purification, and differential inhibition by fluoroquinolones. Antimicrob Agents Chemother 43:1129–36, 1999

88. Pan XS, Fisher LM: DNA gyrase and topoisomerase IV are dual targets of clinafloxacin action in *Streptococcus pneumoniae*. Antimicrob Agents Chemother 42:2810–6, 1998

89. Pestova E, Millichap JJ, Noskin GA, et al: Intracellular targets of moxifloxacin: a comparison with other fluoroquinolones. J Antimicrob Chemother 45:583–90, 2000

90. Pestova E, Beyer R, Cianciotto NP, et al: Contribution of topoisomerase IV and DNA gyrase mutations in *Streptococcus pneumoniae* to resistance to novel fluoroquinolones. Antimicrob Agents Chemother 43:2000–4, 1999

91. Peterson LR: Quinolone molecular structure-activity relationships: what we have learned about improving antimicrobial activity. Clin Infect Dis 15:33SS:S180–6, 2001

92. Piddock LJ, Wise R: The effect of altered porin expression in *Escherichia coli* upon susceptibility to 4-quinolones. J Antimicrob Chemother 18:547–9, 1986

93. Rhee MK, Kowalski RP, Romanowski EG, et al: A laboratory evaluation of antibiotic therapy for ciprofloxacin-resistant *Pseudomonas aeruginosa*. Am J Ophthalmol 138:226–30, 2004

94. Robertson SM, Curtis MA, Schlech BA, et al: Ocular pharmacokinetics of moxifloxacin after topical treatment of animals and humans. Surv Ophthalmol 50(Suppl 1): S32–S45, 2005

95. Rodríguez-Martínez JM, Pascual A, García I, et al: Detection of the plasmid-mediated quinolone resistance determinant qnr among clinical isolates of *Klebsiella pneumoniae* producing AmpC-type beta-lactamase. J Antimicrob Chemother 52:703–6, 2003

96. Saito H, et al: Comparative antimycobacterial activity of Bay 12-8039 and gatifloxacin. Drugs 58(Suppl 2):400–1, 1999

97. Schaefer F, Bruttin O, Zografos L, et al: Bacterial keratitis: a prospective clinical and microbiological study. Br J Ophthalmol 85:842–7, 2001

98. Schaefer S: Methicillin-resistant strains of *Staphylococcus aureus* resistant to quinolones. J Clin Microbiol 27:335–6, 1989

99. Scheld WM, et al: Maintaining fluoroquinolone class efficacy: review of influencing factors. Emerg Infect Dis 9:1651–4, 2003

100. Schlech BA, Wilhelmus KR, Crider JV, et al: Review: Nontuberculous mycobacterial (NTM) infections of the eye and treatment with topical antibacterial agents. Current Med Res Opinion (in press)

101. Schmitz FJ, Higgins PG, Mayer S, et al: Activity of quinolones against Gram-positive cocci: mechanisms of drug action and bacterial resistance. Eur J Clin Microbiol Infect Dis 21:647–59, 2002

102. Seppälä H, Al-Juhaish M, Järvinen H, et al: Effect of prophylactic antibiotics on antimicrobial resistance of viridans streptococci in the normal flora of cataract surgery patients. J Cataract Refract Surg 30:307–15, 2004

103. Shalit I, Berger SA, Gorea A, et al: Widespread quinolone resistance among methicillin-resistant *Staphylococcus aureus* isolates in a general hospital. Antimicrob Agents Chemother 33:593–4, 1989

104. Solomon R, Donnenfeld ED, Perry HD, et al: Penetration of topically applied gatifloxacin 0.3%, moxifloxacin 0.5%, and ciprofloxacin 0.3% into the aqueous humor. Ophthalmology 112:466–9, 2005

105. Speaker MG, Milch FA, Shah MK, et al: Role of external bacterial flora in the pathogenesis of acute postoperative endophthalmitis. Ophthalmology 98:639–49; discussion 650, 1991

106. Stein GE, Schooley S, Kaatz GW: Serum bactericidal activity of the methoxyfluoroquinolones gatifloxacin and moxifloxacin against clinical isolates of *Staphylococcus* species: are the susceptibility breakpoints too high? Clin Infect Dis 37:1392–5, 2003

107. Ta CN, Egbert PR, Singh K, et al: Prospective randomized comparison of 3-day versus 1-hour preoperative ofloxacin prophylaxis for cataract surgery. Ophthalmology 109:2036–40; discussion 2040–1, 2002

108. Takahata M, Shimakura M, Hori R, et al: *In vitro* and *in vivo* efficacies of T-3811ME (BMS-284756) against *Mycoplasma pneumoniae*. Antimicrob Agents Chemother 45:312–315, 2001

109. Takei M, Fukuda H, Kishii R, et al: Target preference of 15 quinolones against *Staphylococcus aureus*, based on antibacterial activities and target inhibition. Antimicrob Agents Chemother 45:3544–7, 2001

110. Tankovic J, Bachoual R, Ouabdesselam S, et al: *In-vitro* activity of moxifloxacin against fluoroquinolone-resistant strains of aerobic Gram-negative bacilli and *Enterococcus faecalis*. J Antimicrob Chemother 43(Suppl B):19–23, 1999

111. Thauvin-Eliopoulos C, Eliopoulos GM: Activity *in vitro* of the quinolones, in Hooper DC, Ethan Rubinstein E (eds): Quinolone Antimicrobial Agents. ASM Press, Washington, DC, 2003, ed 3, chap 5, pp 91–111

112. Thibodeaux BA, Dajcs JJ, Caballero AR, et al: Quantitative comparison of fluoroquinolone therapies of experimental Gram-negative bacterial keratitis. Curr Eye Res 28:337–42, 2004

113. Tran JH, Jacoby GA: Mechanism of plasmid-mediated quinolone resistance. Proc Natl Acad Sci USA 99:5638–42, 2002

114. Tuft SJ, Matheson M: *In vitro* antibiotic resistance in bacterial keratitis in London. Br J Ophthalmol 84:687–91, 2000

115. Tungsiraipat T, Sarayba MA, Kaufman MB, et al: Fluoroquinolone therapy in multiple-drug resistant staphylococcal keratitis after lamellar keratectomy in a rabbit model. Am J Ophthalmol 136:76–81, 2003

116. Wang JC: DNA topoisomerases. Annu Rev Biochem 65:635–92, 1996

117. Watanabe K, Numata-Watanabe K, Hayasaka S: Methicillin-resistant staphylococci and ofloxacin-resistant bacteria from clinically healthy conjunctivas. Ophthalmic Res 33:136–9, 2001

118. Wegener HC, Engberg J: Veterinary use of antimicrobials and impact on human infections, in Hooper DC, Rubinstein E (eds): Quinolone Antimicrobial Agents, 3rd ed. Washington, DC, ASM Press, 2003, pp 387–403

119. Wetzstein HG, Schmeer N, Karl W: Degradation of the fluoroquinolone enrofloxacin by the brown rot fungus Gloeophyllum striatum: identification of metabolites. Appl Environ Microbiol 63:4272–81, 1997

120. Willmott CJ, Maxwell A: A single point mutation in the DNA gyrase A protein greatly reduces binding of fluoroquinolones to the gyrase-DNA complex. Antimicrob Agents Chemother 37:126–7, 1993

121. Wise R: Maximizing efficacy and reducing the emergence of resistance. J Antimicrob Chemother 51(Suppl):S37–S42, 2003

122. Yague G, Morris JE, Pan XS, et al: Cleavable-complex formation by wild-type and quinolone-resistant *Streptococcus pneumoniae* type II topoisomerases mediated by gemifloxacin and other fluoroquinolones. Antimicrob Agents Chemother 46:413–9, 2002

123. Yalvac IS, Basci NE, Bozkurt A, et al: Penetration of topically applied ciprofloxacin and ofloxacin into the aqueous humor and vitreous. J Cataract Refract Surg 29:487–91, 2003

124. Yang SC, Hsueh PR, Lai HC, et al: High prevalence of antimicrobial resistance in rapidly growing mycobacteria in Taiwan. Antimicrob Agents Chemother 47:1958–62, 2003

125. Yoshida H, Bogaki M, Nakamura M, et al: Quinolone resistance-determining region in the DNA gyrase gyrB gene of *Escherichia coli.* Antimicrob Agents Chemother 35:1647–50, 1991

126. Yoshida H, Bogaki M, Nakamura M, et al: Quinolone resistance-determining region in the DNA gyrase gyrA gene of *Escherichia coli.* Antimicrob Agents Chemother 34:1271–2, 1990

127. Zhao X, Drlica K: Restricting the selection of antibiotic-resistant mutants: a general strategy derived from fluoro— quinolone studies. Clin Infect Dis 33(Suppl 3):S147–S156, 2001

ELSEVIER

Ocular Pharmacokinetics of Moxifloxacin After Topical Treatment of Animals and Humans

Stella M. Robertson, PhD, Michael A. Curtis, PhD, Barry A. Schlech, PhD,
Andrew Rusinko, PhD, Geoffrey R. Owen, PhD, Olga Dembinska, PhD,
John Liao, PhD, and David C. Dahlin, PhD

Alcon Research, Ltd., Fort Worth, Texas, USA

Abstract. The ocular penetration and pharmacokinetics of moxifloxacin in comparison to other fluoroquinolones (ofloxacin, ciprofloxacin, gatifloxacin, norfloxacin, levofloxacin, and lomefloxacin) have been determined by *in vitro* and *ex vivo* techniques, as well as in animal and human studies. This article reviews the original pharmacokinetics work performed by Alcon and other studies reported in the ocular fluoroquinolone literature. The results consistently demonstrate higher maximum concentrations for moxifloxacin relative to the other fluoroquinolones in ocular tissues with levels well above its minimum inhibitory concentrations for relevant ocular pathogens. This superior performance is due to the unique structure of moxifloxacin that combines high lipophilicity for enhanced corneal penetration with high aqueous solubility at physiological pH. The latter property creates a high concentration gradient at the tear film/corneal epithelial interface providing a driving force for better ocular penetration for moxifloxacin. In addition, the higher concentration of moxifloxacin in VIGAMOX® (i.e., 0.5% vs. 0.3%) allows more antibiotic to be available to ocular tissues. It is clear from the array of studies summarized in this report that moxifloxacin penetrates ocular tissues better (two- to three-fold) than gatifloxacin, ciprofloxacin, ofloxacin, or levofloxacin. This consistent, enhanced penetration of topical moxifloxacin offers powerful advantages for ophthalmic therapy. (Surv Ophthalmol 50:S32–S45, 2005. © 2005 Elsevier Inc. All rights reserved.)

Key words. fluoroquinolones · moxifloxacin · ocular penetration · ophthalmic therapy · pharmacokinetics · VIGAMOX®

Introduction

Moxifloxacin is a novel fourth-generation fluoroquinolone with high potency against both gram-positive and gram-negative bacterial pathogens. It has the highest potency in its class against *Staphylococcus aureus* and *Staphylococcus epidermidis*.[10] Moxifloxacin has been developed as a 0.5% solution for topical, ocular use as moxifloxacin ophthalmic solution 0.5%, (VIGAMOX®, Alcon Laboratories, Inc., Fort Worth, TX). In addition to high potency, a desirable characteristic of topical antibiotics is their rapid migration across the cornea and extensive penetration into anterior ocular tissues. The ocular bioavailability of different antibiotics can be compared on the basis of the concentration achieved in the tears, cornea, conjunctiva, aqueous, or vitreous humor. The concentration of the antibiotic in these tissues should be maintained for sufficient time above the minimum

S32

inhibitory concentration (MIC) for important pathogens in order to achieve effective bacterial killing. The higher the concentration above the antibiotic's MIC, the greater the protection against infection. Alcon and other investigators have conducted numerous non-clinical and clinical studies to measure the ocular uptake and pharmacokinetics (PK) of moxifloxacin in comparison to other topical fluoroquinolones. The results of these recent *in vitro, ex vivo, in vivo,* and human clinical studies are reviewed in this article.

Materials and Methods

IN VITRO/EX VIVO METHODS

For most of the *in vitro* or *ex vivo* corneal penetration experiments, fresh, excised corneas from rabbits were mounted in corneal perfusion chambers. Solutions of fluoroquinolones or commercial products were applied to the epithelial side of the excised corneas for a period of time (e.g., 5 minutes) and the amount of antibiotic crossing the cornea was measured on the endothelial side. Dembinska and colleagues (F1) measured the effects of topical antibiotic preparations on the corneal epithelial barrier function and also measured the rate of carboxyfluorescein or sodium fluorescein permeation through the treated excised corneas. In this study, the permeation of each dye was assessed by linear regression fitted to the ascending part of its curves. Statistical analysis was performed on individual slopes (n ≥ 4) and included the mean, the standard deviation, and one-way ANOVA and Tukey tests for multiple comparisons. Each analysis included controls pooled from both fluoroquinolones. In other *in vitro* studies canine kidney (MDCK) cells were used as a model to assess and compare tissue penetration properties for various fluoroquinolone formulations (F2). For these studies, linear regressions were determined using S-Plus 6.0 for Windows, Release 2, from the Insightful Corporation.

IN VIVO ANIMAL STUDIES

For *in vivo* rabbit studies, animals were treated topically with various fluoroquinolone formulations and euthanized at the appropriate time-points following topical treatment. The ocular tissues were analyzed for fluoroquinolone content. Aqueous humor,

vitreous humor, tear film, and plasma samples were analyzed using aliquots of fixed volume while other tissues (i.e., cornea, iris-ciliary body, conjunctiva) were weighed and homogenized in water prior to analysis.

HUMAN CLINICAL STUDIES

For human studies, aqueous and vitreous humor samples were obtained using a syringe and canula during cataract or other ophthalmic surgeries. Also, samples of conjunctiva were excised from normal human volunteers after topical instillation of fluoroquinolone preparations[15] (F3).

ANALYSIS OF SAMPLES

For all studies, tissue samples were generally stored in sealed vials at −20°C or colder until analyzed. Concentrations of moxifloxacin and other fluoroquinolones were determined using high performance liquid chromatography (HPLC) with fluorescence detection or HPLC/tandem mass spectrometry. Concentrations were normally given as μg/ml or μg/g of tissue. Lower limits of quantitation ranged from 2–30 ng/g or ng/mL depending on the ocular tissue.

DOSING REGIMENS

Dosing regimens for animal and human studies varied according to the test protocol. The dosing details of the *in vivo* and clinical phases of various studies are discussed in the sections below or given in the Tables.

Results

IN VITRO/EX VIVO STUDIES

The corneal penetration and permeability characteristics of moxifloxacin were investigated in a series of Alcon *in vitro* and *ex vivo* studies that are summarized below.

Ocular *Ex Vivo* Penetration and Corneal Permeability of Moxifloxacin and Gatifloxacin

One Alcon study (F4) involved exposing the epithelial side of excised corneas from New Zealand white rabbits to 0.004% (0.1 mM) solutions of moxifloxacin and gatifloxacin in BSS balanced salt solution. The concentrations of the fluoroquinolones were determined by HPLC of the perfusates over a 5-hour period. The results in Table 1 show a 3.6-fold

F1 Dembinska O, Stout KR, Podval J, Rodeheaver DP: Corneal epithelial barrier function following the exposure to VIGAMOX® and Zymar in *ex vivo* model of corneal penetration. Invest Ophthalmol Vis Sci 46: E-Abstract 4901, 2005.

F2 Rusinko A, May J, Liao J, Namil A, Hellberg M: A study of the enhanced corneal penetration of moxifloxacin. Invest Ophthalmol Vis Sci 45: E-Abstract 4907, 2004.

15 Wagner RS, Abelson MB, Shapiro A, Torkildsen G: Evaluation of fluoroquinolone antibiotic concentrations in human conjunctival tissue following topical administration. Invest Ophthalmol Vis Sci 46: E-Abstract 4888, 2005.

TABLE 1

In Vitro/Ex Vivo Penetration Study for Moxifloxacin and Gatifloxacin (F4)

	Moxifloxacin[*]	Gatifloxacin[*]	Difference
Corneal permeability to FQ ($\times 10^{-7}$ cm/sec $\pm$ SD)	91 $\pm$ 9	25 $\pm$ 2	moxifloxacin penetrated 3.6 $\times$ more than gatifloxacin (P = 0.005)[**]
Time before appearance of FQ on endothelial side of cornea (min $\pm$ SD)	49 $\pm$ 1	99 $\pm$ 12	moxifloxacin penetrated 2.0 $\times$ faster than gatifloxacin (P = 0.02)[**]

[*] Test articles were 0.004% solutions of fluoroquinolone in a preservative-free vehicle and each mean value was the average of three corneas $\pm$ Standard deviation.
[**] Significance determined with paired t-test.

better penetration (i.e., higher corneal permeability) for moxifloxacin versus gatifloxacin. In addition, the time before the appearance of antibiotic on the endothelial side was about two-fold earlier (i.e., quicker penetration) for moxifloxacin than for gatifloxacin. In this study, none of these solutions contained benzalkonium chloride (BAC) as a preservative.

Another Alcon study (F1, F4) used two commercial antibiotic products and determined the corneal permeability to carboxyfluorescein (CF) or sodium fluorescein (SF) as a measure of corneal integrity. In this *ex vivo* permeability study, the commercial fluoroquinolone preparations (VIGAMOX® and Zymar® [gatifloxacin ophthalmic solution 0.3%; Allergan, Inc, Irvine, CA]) were applied to the epithelial surface of the excised cornea of the rabbit for 5 minutes. After rinsing, corneas were exposed to either CF or SF for 5 minutes and the perfusate collected over 2 hours. The level of CF or SF in the perfusate was measured by spectrophotometry and the more CF or SF found in the perfusate then the more corneal damage occurred. The results in Table 2 show again that moxifloxacin penetrates the cornea better than gatifloxacin (6.5 vs 2.8 μg/min) and faster (11 vs. 18 min). Also, CF and SF passed through the cornea faster after treatment with Zymar than after treatment with VIGAMOX® (2.77 vs 1.83 pMol/ml/min for CF, 0.50 vs. 0.28 pMol/ml/min for SF). Although moxifloxacin penetrates the cornea faster and accumulates at higher concentrations in the aqueous humor than gatifloxacin (Table 1 and 2), VIGAMOX® maintained the corneal integrity better than Zymar®. There was a 1.6-fold lower accumulation of CF (37 vs. 60 pMol/ml) after treatment with VIGAMOX® than with Zymar® (Table 2). The presence of benzalkonium chloride in Zymar is likely causing a loss of integrity to the intercellular (gap)

junctions in the corneal epithelium, whereas VIGAMOX® has an advantage in this regard (i.e., it is self-preserved and contains no benzalkonium chloride) (F4). These results indicate that the enhanced corneal penetration of moxifloxacin relative to gatifloxacin is due to inherent differences in molecular structure, the higher fluoroquinolone concentration (0.5% vs. 0.3%), and the lack of BAC in the commercial preparation.

Lipophilicity and MDCK Cell Permeability of Fluoroquinolones (Fig. 1)

Lipophilicity and aqueous solubility are two factors that govern corneal penetration rates. The purpose of this Alcon study (F2) was to identify which molecular properties are responsible for the superior bioavailability of topically applied moxifloxacin in comparison to six other fluoroquinolones. Secondly, a mathematical model was developed to predict corneal permeability of fluoroquinolone antibiotics based upon their *in vitro* data using Madin-Darby canine kidney (MDCK) cells and physiochemical properties. For the MDCK studies, 10 μM solutions of fluoroquinolones were tested. Aqueous solubility and lipophilicity values were determined for each fluoroquinolone. The permeabilities of seven fluoroquinolones (i.e., moxifloxacin, ciprofloxacin, norfloxacin, ofloxacin, levofloxacin, lomefloxacin, and gatifloxacin) were determined in the MDCK model and the *in vitro* corneal penetration data published earlier by Fukada.[5] The results of these comparisons are shown in Table 3. The MDCK permeability showed a high correlation with lipophilicity ($R^2 = 0.92$ see Fig. 1) and corneal permeability ($R^2 = 0.93$) indicating that the *in vitro* MDCK model is an excellent predictor of corneal penetration. Moxifloxacin had the highest molecular weight (401.4) and showed higher lipophilicity (0.24), greater aqueous solubility (>6.43%) and the best MDCK and corneal

F4   Owen GR, Dembinska O, Stout KR, Mendiola MK: Corneal penetration and changes in corneal permeability of moxifloxacin versus gatifloxacin. Invest Ophthalmol Vis Sci 45: E-Abstract 4910, 2004.

TABLE 2

In Vitro/Ex Vivo Permeability Studies with Two Commercial Topical Fluoroquinolones (F1, F4)

	Moxifloxacin* mean ± SD	Gatifloxacin* mean ± SD	Control (Balanced Salt Solution) mean ± SD	Difference**
Rate of accumulation of FQ in the cornea; value in µg/min***	6.5 ± 1.6	2.8 ± 0.3	Not Applicable	moxifloxacin penetrated 2.3 × more than gatifloxacin
Lag time before FQ appearance in the cornea; time in minutes	11 ± 4	18 ± 5	Not Applicable	moxifloxacin was 1.6 × faster in getting to the cornea
Rate of carboxyfluorescein (CF) permeation (slope in pMol/mL/min)	1.83 ± 0.68	2.77 ± 1.19	0.71 ± 0.33	Zymar caused CF to penetrate 4 × more than control (P < 0.001) and 1.5 × more than VIGAMOX® (P < 0.05)
Rate of sodium fluorescein (SF) permeation (slope in pMol/mL/min)	0.28 ± 0.13	0.50 ± 0.17	0.35 ± 0.16	Zymar caused SF to penetrate 1.8 × more than VIGAMOX® (P < 0.05)
Peak carboxyfluorescein (CF) accumulation (pMol/mL)	37 ± 9	60 ± 5	18 ± 8	Zymar caused CF to penetrate 1.6 × more than VIGAMOX® (P < 0.05)

* Test articles were the commercial preparation of moxifloxacin 0.5% (VIGAMOX®) and gatifloxacin 0.3% (Zymar®). Each mean value was the average of four corneas

† standard deviation.

** Significance determined with paired *t*-test where indicated.

*** Minutes as $\log_e$ scale.

permeability (35.2 and 15.8, respectively) than any of the other six fluoroquinolones tested (F2).

IN VIVO ANIMAL STUDIES

Numerous *in vivo* studies conducted in rabbits have characterized the ocular penetration and pharmacokinetics of moxifloxacin and other fluoroquinolones as comparators.

Ocular Penetration of 0.3% Solutions of Moxifloxacin and Ofloxacin

This Alcon study (F5) was designed to measure the ocular penetration and distribution of moxifloxacin and ofloxacin into the aqueous humor, cornea, iris-ciliary body, tear film, and plasma following a single topical ocular administration of 0.3% solutions to rabbits. The eyes of male Dutch-belted rabbits were dosed with a single drop (30µl) of either 0.3% moxifloxacin or 0.3% ofloxacin solutions. The moxifloxacin 0.3% solution tested was prepared as a 0.3% solution in phosphate buffer saline (pH 7.4); the commercial product (VIGAMOX®) contains a higher concentration of moxifloxacin, namely, 0.5%. The 0.3% ofloxacin solution was the commercially available Ocuflox® (pH 6.4; Allergan, Irvine, CA). Aqueous humor, cornea, iris-ciliary body, tear film, and plasma were collected up to 48 hours post-dose for analyses of drug concentrations by reverse phase HPLC. The corneas from the animals were not altered in any way to increase absorption. The investigators were careful to ensure the integrity of the corneal epithelium in the study. The in-life work was conducted at a high-quality independent GLP research laboratory. All animals underwent an examination with sodium fluorescein prior to dosing to verify the integrity of the cornea. This examination was documented for every eye in the study. The study directions specified that extreme care be taken to avoid any injury to the corneas in all groups throughout the study. The moxifloxacin and ofloxacin concentrations achieved are given for the various times post-dose in Table 4 for aqueous humor, cornea, iris-ciliary body and plasma. The moxifloxacin concentrations were typically higher than ofloxacin in all tissues tested and at most time pulls. The maximum concentration (Cmax) for moxifloxacin was two-fold higher than ofloxacin in the cornea and 3.5-fold higher in the aqueous humor. Moxifloxacin's Cmax for iris-ciliary body and plasma were 2.5- and 1.5-fold

F5 Robertson SM, Sanders M, Jasheway D, Trawick D, Veltman J, Hamner S, Schlech BA, Hilaski R, Dahlin DC: Penetration and distribution of moxifloxacin and ofloxacin into ocular tissues and plasma following topical ocular administration to pigmented rabbits. Invest Ophthalmol Vis Sci 44: E-Abstract 1454, 2003.

TABLE 3

Molecular Properties of Fluoroquinolones (F2)

Fluoroquinolone	Molecular Weight	Aqueous solubility (%)	MDCK Permeability (cm/s) × 10⁷	Corneal Permeability* (cm/s) × 10⁷	Lipophilicity C-7, π
Moxifloxacin	401.4	>6.43**	35.2	15.8	0.24
Gatifloxacin	375.4	0.21	10.3	4.6	0.11
Ciprofloxacin	331.3	0.02	4.5	2.46	−0.35
Ofloxacin	361.4	0.35	15.1	6.78	0.06
Norfloxacin	319.3	0.05	3.3	1.63	−0.35
Levofloxacin	361.4	1.85**	16.4	6.95	0.06
Lomefloxacin	351.3	0.13	6.6	3.58	0.11

* Data for moxifloxacin and gatifloxacin were estimated by linear regression; Data for remaining five fluoroquinolones were from Fukada and Sasaki.[5]
** After 13 weeks of mixing.

higher than ofloxacin's Cmax. Ofloxacin concentrations in the cornea fell below its MIC_{50} of 0.5 μg/ml for methicillin-resistant *Staphylococcus aureus* (MRSA) by 8 hours while the moxifloxacin concentration (0.25 μg/ml) was about four-fold higher than its MIC_{50} (0.06 μg/ml) for MRSA even at 48 hours after instillation. In aqueous humor, ofloxacin concentrations fell below the quantitation limit of detection (i.e., 33 ng/g) after 4 hours, whereas there were still measurable amounts of moxifloxacin found at the last time point of 48 hours. The moxifloxacin iris-ciliary body concentrations were substantially higher than those for ofloxacin. These results demonstrate that moxifloxacin has a better penetration profile compared to ofloxacin. Results from other Alcon studies (unpublished Alcon data) indicate that ofloxacin has a better penetration profile than ciprofloxacin. Following single-drop instillation of 0.3% ciprofloxacin solution, the mean ciprofloxacin concentration at 1 hour was 7.3-fold lower than that for ofloxacin (0.076 μg/g vs. 0.555 μg/g, respectively). Based on the results of these moxifloxacin, ofloxacin, and ciprofloxacin single-drop studies, it can be concluded that the penetration profile of moxifloxacin into the aqueous humor and cornea is greater than ofloxacin, which is greater than that for ciprofloxacin. Also maximal plasma concentrations of both moxifloxacin and ofloxacin were low (<0.01 μg/ml) and declined rapidly in the plasma. The moxifloxacin plasma concentration in this study was 700-fold lower than the tolerated doses from toxicology studies and 300-fold lower than those measured in clinical studies at oral doses. Even at these high oral doses, there were no relevant deviations from normal biochemical or hematological parameters. These results demonstrate a wide margin of safety for topical ophthalmic use of 0.3% moxifloxacin solutions.

Tear Film Concentrations of Moxifloxacin and Ofloxacin (Fig. 2)

In this Alcon study (F5), nine male Dutch-Belted rabbits were used to measure the tear film concentra-

tions achieved after a single drop of 0.3% moxifloxacin or 0.3% ofloxacin solutions. Each animal, except the undosed controls (n = 3/group), received a single 30-μl topical ocular dose to right (OD) eye using a calibrated positive displacement pipettor. Tear film samples (0.5–1.0 μl) were collected from the dosed eyes using capillary tubes at 1, 2, 3, 5, 60, 90, 120, 180, 240, and 360 minutes. Samples were weighed and stored at −70°C prior to analysis by reverse phase HPLC. The results of this single-drop study are shown in Fig. 2 and indicate that at the early time pulls, the concentrations were similar, but over the course of the next hour, ofloxacin concentration dropped more rapidly than moxifloxacin with moxifloxacin levels being about seven-fold to 10-fold higher than ofloxacin. Ofloxacin was not detectable in the tear film (i.e., <0.5 μg/ml) beyond 90 minutes whereas moxifloxacin remained at concentrations at or above 1.0 μg/ml up to the last time point of 6 hours.

Effect of Cataract Surgery on Ocular Levels of Moxifloxacin

Another study by Mather and colleagues[12] at the Proctor Foundation mimicked cataract surgery in Dutch-belted rabbits. The moxifloxacin concentrations in the aqueous and vitreous humors were measured 30, 60, and 120 minutes after topical instillation of moxifloxacin ophthalmic solution 0.5%. Mean tissue concentrations obtained in surgical eyes were compared with concentrations obtained in non-surgical eyes. Moxifloxacin concentrations were determined by HPLC. A total of six drops of moxifloxacin ophthalmic solution 0.5% was administered to each eye, as follows: 60 minutes before surgery three single drops (30 μl/drop) were applied separated by 5 minutes. The same regimen of three drops (each separated by 5 minutes) was applied at the end of surgery. The results of this study are shown in Table 5. This multi-drop regimen of moxifloxacin ophthalmic

TABLE 4

Animal Study: Fluoroquinolone Concentration in Aqueous Humor and Cornea Following a Single Topical Ocular Dose of 0.3% Solutions of Moxifloxacin and Ofloxacin to Dutch-belted Rabbits (F5)

	Time (hr)	Moxifloxacin Mean Conc (μg/mL) ± SD	Ofloxacin (μg/g) Mean Conc (μg/mL) ± SD
Aqueous humor	0.5	1.78 ± 0.39[*]	0.507 ± 0.489[*]
	1	0.993 ± 0.075	0.267 ± 0.134
	2	0.304 ± 0.059	0.229 ± 0.031
	4	0.0589 ± 0.0071	0.0933 ± 0.0589
	8	0.0353 ± 0.0232	BLQ
	12	0.0207 ± 0.0002	BLQ
	24	0.0182 ± 0.0029	BLQ
	48	0.0172 ± 0.0099	BLQ
Cornea	0.5	12.5 ± 3.8[*]	6.02 ± 2.27[*]
	1	5.89 ± 0.78	2.34 ± 0.99
	2	2.02 ± 0.13	2.41 ± 0.46
	4	0.65 ± 0.082	1.05 ± 0.39
	8	0.99 ± 0.288	0.493 ± 0.123
	12	0.437 ± 0.225	0.150 ± 0.042
	24	0.253 ± 0.119	0.270 ± 0.240
	48	0.247 ± 0.015	0.0967 ± 0.0889
Iris-ciliary body	0.5	6.26 ± 2.07	0.800 ± 0.360
	1	10.4 ± 5.6	0.653 ± 0.423
	2	8.54 ± 1.45	4.43 ± 1.99
	4	11.0 ± 1.7	5.42 ± 2.88[*]
	8	13.5 ± 4.7[*]	4.36 ± 1.57
	12	9.42 ± 3.76	2.92 ± 0.07
	24	10.7 ± 6.2	3.33 ± 1.01
	48	7.68 ± 2.14	2.78 ± 0.33
Plasma	0.5	0.0130 ± 0.0016[*]	0.0069 ± 0.0087
	1	0.0109 ± 0.0010	0.0080 ± 0.0010[*]
	2	0.0065 ± 0.0003	0.0038 ± 0.0003
	4	BLQ	BLQ
	8	BLQ	BLQ
	12	BLQ	BLQ
	24	BLQ	BLQ
	48	BLQ	BLQ

BLQ = Below Limit of Quantitation of 33 ng/g.

Note: Each mean value was average of three samples;

[*] designates the maximum concentration obtained for that antibiotic in that tissue.

solution 0.5% produced aqueous humor concentrations of 12.2 to 32.6 μg/ml that were well above the MICs of even resistant strains of the most common organisms implicated in post cataract surgery endophthalmitis (e.g., *Staphylococcus aureus* and coagulase negative *Staphylococcus*). Relatively high concentrations of moxifloxacin were detected in the aqueous humor of both groups at all time points. At all time points, the mean moxifloxacin concentrations are at least 200-fold greater than the MIC$_{50}$ value for FQ-susceptible *Staphylococcus* and at least four-fold higher than MIC$_{50}$ for FQ-resistant *Staphylococcus*. There were no statistically significant differences between surgical or nonsurgical eyes at any time-point, indicating that cataract surgery does not alter the penetration of moxifloxacin into aqueous or vitreous humor. However, the study demonstrated that topical moxifloxacin administered as three single drops, 60

minutes prior to surgery and immediately after surgery resulted in a mean aqueous concentration for moxifloxacin that was 200-fold higher than the median MIC for fluoroquinolone-susceptible isolates of *Staphylococcus aureus* or coagulase-negative *Staphylococcus*.

Ocular Penetration of 0.5% Moxifloxacin, 0.3% Ofloxacin, and 0.3% Gatifloxacin

This Alcon rabbit study (F6) measured the ocular pharmacokinetics of topical moxifloxacin 0.5% (VIGAMOX®), ofloxacin 0.3% (Ocuflox®), and gatifloxacin 0.3% (Zymar®) solutions following repeated

[F6] Robertson SM, Sanders M, Jasheway D, et al: Absorption and distribution of moxifloxacin, ofloxacin and gatifloxacin into ocular tissues and plasma following topical ocular administration to pigmented rabbits. Invest Ophthalmol Vis Sci 45: E-Abstract 4906, 2004.
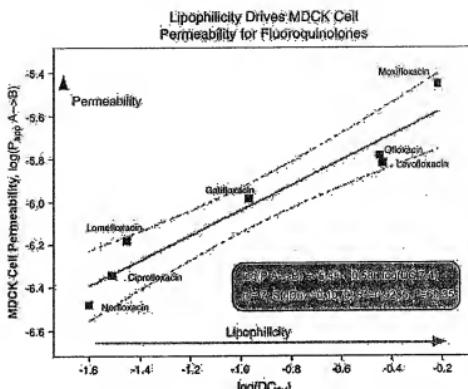
Fig. 1. Comparison of lipophilicity versus MDCK cell permeability (F2).

topical ocular doses of three times per day for 3 days to pigmented rabbits (F6). Male Dutch-belted rabbits received a thorough slit-lamp biomicroscopic examination prior to dosing. Only rabbits with no ocular defects were randomized into the three test groups. There were 30–33 rabbits per group. Each rabbit received bilateral 30-μl doses of VIGAMOX®, Ocuflox®, or Zymar® three times per day for 5 days. Doses were administered at 8 AM, 12 PM, and 4 PM on days 1 and 2; at 8 AM, 4 PM, and 12 AM on day

3; and at 8 AM on day 4. Prior to euthanasia, blood from each animal was collected and centrifuged for the plasma fraction. Three treated animals per time point from each group (three eye pairs or n = 6 eyes) were euthanized at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 144, and 288 hours, followed by collection of aqueous humor, cornea, iris-ciliary body, and vitreous humor (0.25 hour was excluded from the latter two tissues). Sample weights were recorded and all samples were frozen on dry ice prior to storage at −70°C.

*Ofloxacin levels at 90 minutes were below the quantitation limit (0.5 ug/ml.)
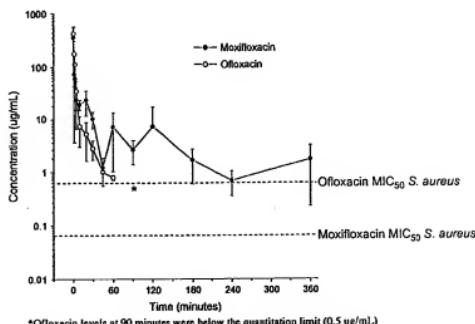
Fig. 2. Mean concentrations of fluoroquinolones in tear film of rabbits after a single topical dose of moxifloxacin or ofloxacin (F5).

TABLE 5

Animal Study: Effect of Cataract Surgery on Aqueous and Vitreous Humor Concentrations
of Moxifloxacin in Rabbits[12]

	Time [hr]	Moxifloxacin in Aqueous Humor Mean Conc [μg/mL] ± SD	Moxifloxacin in Vitreous Humor Mean Conc [μg/mL] ± SD
Surgical group	30 min	13.9 ± 7.2	0.0668 ± 0.0592
	60 min	16.2 ± 3.12	0.0666 ± 0.0863
	120 min	12.2 ± 5.1	0.400 ± 1.0833
Nonsurgical group	30 min	25.3 ± 16.8	0.0431 ± 0.1227
	60 min	32.6 ± 20.1	0.2000 ± 0.631
	120 mm	15.7 ± 15.0	0.0544 ± 0.0497

n = 18 eyes per arm; differences in moxifloxacin concentration in eyes between the surgical and nonsurgical group were compared using a Wilcoxon signed rank test. An alpha of 0.05 was used to determine statistical significance. There were no significant differences between surgical and nonsurgical groups (P > 0.68).

The analysis of drug concentrations in the tissues was performed by a reverse phase HPLC/fluorescent method. Ocular tissues were homogenized in water and moxifloxacin, ofloxacin, and gatifloxacin were isolated by extraction methods. Samples were analyzed by reverse phase liquid chromatography. Lower limits of quantitation were: aqueous humor (0.0025 μg/ml for all FQs), cornea (0.0174 μg/g for all FQs), iris-ciliary body (0.218 μg/g for moxifloxacin and ofloxacin; 0.0992 μg/g for gatifloxacin), vitreous humor (0.00041 μg/g for all FQs), and plasma (0.0005 μg/ml for all FQs). The results are presented in Table 6 and indicate that, as expected, all three fluoroquinolones penetrated into the eye. In all ocular tissues, moxifloxacin achieved the greatest maximal concentrations compared to ofloxacin and gatifloxacin. Gatifloxacin penetrated the least. Moxifloxacin achieved levels that were four- to five-fold more than gatifloxacin and two- to four-fold more than ofloxacin. All three compounds showed prolonged retention in the pigmented iris-ciliary body due to melanin binding, as expected for fluoroquinolones. These results show that moxifloxacin is well absorbed into the anterior ocular tissues and can be

found in the vitreous humor after topical ocular application to a level greater than either ofloxacin or gatifloxacin (F6).

Penetration of Moxifloxacin and Gatifloxacin into Rabbit Aqueous Humor

The purpose of this published study by Levine[11] from the University of Arizona was to evaluate the aqueous penetration of the fourth-generation fluoroquinolones, moxifloxacin and gatifloxacin. Twenty New Zealand white rabbits were divided into two groups and dosed with commercial topical preparations of moxifloxacin (VIGAMOX®) or gatifloxacin (Zymar®). Group 1 [keratitis protocol] received a dose every 15 minutes for 4 hours and the aqueous humor was sampled 10 minutes after last dose; group 2 (cataract prophylaxis protocol) was dosed four times a day for 10 days and the aqueous humor was sampled 1 hour after the last dose in 12 eyes and 24 hours after the last dose in 8 eyes. The concentrations of FQ in the aqueous samples were determined by HPLC. Results are shown in Table 7. The keratitis-dosing regimen produced mean moxifloxacin concentrations of 11.06 μg/ml that was significantly

TABLE 6

Animal Study Fluoroquinolone Concentrations [μg/mL or μg/g]* in Five, Rabbit Tissues Following Multiple** Topical Ocular Dosing of VIGAMOX®, Zymar®, or Ocuflox® (F6)

	Moxifloxacin		Ofloxacin		Gatifloxacin	
Aqueous humor	1.42 ± 0.60	30 min	0.405 ± 0.135	30 min	0.310 ± 0.075	60 min
Cornea	21.3 ± 8.6	15 min	8.01 ± 2.79	15 min	4.9 ± 0.70	15 min
Iris-ciliary body	35.0 ± 6.6	120 min	10.0 ± 3.0	120 min	12.6 ± 3.3	480 min
Vitreous humor	15.6 ± 18.4	60 min	3.27 ± 5.36	240 min	2.79 ± 3.64	60 min
Plasma	11.5 ± 2.2	30 min	9.59 ± 2.41	30 min	6.80 ± 1.74	30 min

* Maximum concentrations (Cmax) were measured in μg/mL for the aqueous and vitreous humors and plasma, and in μg/g for the corneas, and iris-ciliary bodies.
** Dosed three times a day for 3 days plus one drop on the 4th day [10 drops total]; three treated animals per time point from each group (i.e., three eye pairs or n = 6 eyes); samples were taken at 10–11 timepoints; there were a total of 30–33 rabbits dedicated to each treatment group.

TABLE 7

Animal Study: Mean Aqueous Humor Concentration of Moxifloxacin and Gatifloxacin Following Two
Topical Ocular Dosing Regimens in Rabbits[11]

	Moxifloxacin				Gatifloxacin			
Dosing Protocol	n	Mean Concentration (μg/mL $\pm$ SD)	SEM	Range	n	Mean Concentration (μg/mL $\pm$ SD)	SEM	Range
Keratitis[*]	9	11.06 $\pm$ 3.55	1.18	7.66–18.87	8	7.57 $\pm$ 2.22	0.78	4.75–10.86
Prophylaxis[**]	6	1.75 $\pm$ 1.17	0.48	0.92–3.87	6	1.21 $\pm$ 0.72	0.29	0.44–2.14

SD = standard deviation; SEM = Standard error of the mean. Statistical analyses of the aqueous antibiotic concentrations
was performed using a 2-tailed t test assuming equal variances.
   [*] Moxifloxacin concentration significantly higher than gatifloxacin (P = 0.030) in the keratitis dosing regimen.
   [**] No difference between concentrations of the two antibiotics (P = 0.359) in the prophylaxis dosing regimen.

higher (p = 0.03) than the mean concentration for
gatifloxacin of 7.57 μg/ml. The 10-day cataract pro-
phylaxis regimen yielded similar results of 1.75 μg/ml
for moxifloxacin and 1.21 μg/ml for gatifloxacin.

HUMAN CLINICAL STUDIES

Ocular penetration of moxifloxacin in humans was
assessed in patients undergoing ocular surgery by
monitoring drug concentrations in samples of aque-
ous humor or vitreous humor. Conjunctival levels
were also determined in a study in healthy volunteers.

Penetration into Human Aqueous Humor

Moxifloxacin and Gatifloxacin (Cataract)

In studies by Katz et al[8], (F7) (Krieger Eye Insti-
tute), cataract patients were randomized to one of two
groups (30 patients per group) (F7). For regimen 1,
patients received moxifloxacin ophthalmic solution
0.5% as one drop to the operative eye four times at
15-minute intervals on the day of surgery. For regi-
men 2, the patients received moxifloxacin ophthal-
mic solution 0.5% as one drop to the operative eye
four times at 15-minute intervals on the day prior
to surgery plus four additional doses at 15-minute
intervals on the day of surgery (F7). One sample per
subject was collected at one of the following time-
points: 0.25, 0.5, 1, 2, or 3 hours after the last dose. This
unique study design allowed researchers to calculate
concentrations over time, to estimate an area under
the inhibitory curve (AUIC). Table 8 gives the com-
parative aqueous humor values for patients treated
with VIGAMOX® or Zymar using this approach. The
moxifloxacin aqueous humor Cmax was 2.3- to 3.1-
fold higher than gatifloxacin and the AUC$_{0-3h}$ was

F7 Katz HR, Lane S, Masket S, Sall K, Orr S, Faulkner R, Robert-
son SM, Dahlin DC: Human aqueous concentrations of moxiflox-
acin and gatifloxacin following two multiple-dose topical ocular
dosing regimens with VIGAMOX® and Zymar. Invest Ophthalmol
Vis Sci 46: E-Abstract 4907, 2005.

significantly (P < 0.05) higher (>2-fold) than gati-
floxacin for both regimens. These C$_{max}$ values (1.55–
1.61 μg/ml) were 25- to 30-fold above the median
MIC for Staphylococcus aureus and Staphylococcus epider-
midis isolates from clinical cases of endophthalmitis.
Moxifloxacin concentrations were 16–20 times above
the MIC for these organisms even 3 hours after the last
dose. There was no statistically significant difference
between regimen 1 and regimen 2 (p > 0.05). The
findings showed that the ocular penetration of moxi-
floxacin is at least two-fold greater than gatifloxacin
with either regimen.

Kim et al[9] administered VIGAMOX® or Zymar®
topically to the eyes of 25 cataract patients prior to
their surgeries.[9] Dosage was one drop every 10 mi-
nutes for four doses beginning one hour before sur-
gery. At the time of surgery, a 30-gauge cannula on
a tuberculin syringe was used to acquire the speci-
mens of aqueous humor. Antibiotic concentrations
were determined by HPLC. The mean concentration
of moxifloxacin in the aqueous humors was 1.80 $\pm$
1.21 μg/mL while that for gatifloxacin was 0.48 $\pm$ 0.34
(Table 9). This three-fold difference in antibiotic
concentration favoring moxifloxacin was statistically
significant (P = 0.00003).

Moxifloxacin (Vitrectomy)

In another clinical study, Hariprasad et al[7] deter-
mined moxifloxacin concentrations in aqueous
humors of 20 vitrectomy patients following topical
administration of moxifloxacin ophthalmic solution
0.5% for 3 days at 2- or 6-hour intervals. Assays were
performed using HPLC. The mean moxifloxacin
concentrations in the aqueous humor are shown in
Table 9. The mean concentrations were 2.28 and
0.88 μg/ml for the 2- and 6-hour intervals, respec-
tively for the aqueous humor. These were sig-
nificantly different (P = 0.01, t-test, two-tailed
homoscedastic function). The moxifloxacin MIC$_{90}$s
were far exceeded for a wide spectrum of pathogens

TABLE 8

Human Study: Moxifloxacin and Gatifloxacin Penetration into Aqueous Humor
via Two Treatment Regimens[8] (F7)

Time	Moxifloxacin		Gatifloxacin	
	Regimen 1	Regimen 2	Regimen 1	Regimen 2
Cmax (μg/mL) ± SD	1.55 ± 0.86	1.61 ± 0.71	0.74 ± 0.66	0.91 ± 0.54
Tmax (min)	30	120	30	60
AUC0-5 (μg/mL) ± SE	2.99 ± 0.28	3.97 ± 0.44	1.79 ± 0.21	1.58 ± 0.23

SE = standard error; SD = standard deviation.
Regimen 1: Dosed 1 drop every 15 min for four doses prior to surgery [four drops total].
Regimen 2: Dosed 1 drop every 15 min for four doses prior to surgery plus four doses the day prior to surgery [eight drops total].
n = 30 for each of the four treatment groups; 120 aqueous humor samples were collected, 60 from patients randomized to each fluoroquinolone with 30 at each dosing regimen.
Moxifloxacin regimen 1 was significantly better than gatifloxacin regimen 1 (P < 0.05).
Moxifloxacin regimen 2 was significantly better than gatifloxacin regimen 2 (P < 0.05).

including *Staphylococcus epidermidis, S. aureus, Streptococcus pneumoniae, S. pyogenes, Propionibacterium acnes, Haemophilus influenzae,* and *Escherichia coli.*

Moxifloxacin and Gatifloxacin (Phacoemulsification)

McCulley, Aronowicz and co-investigators at the University of Texas Southwestern Medical Center determined the aqueous humor concentrations of moxifloxacin and gatifloxacin after five topical doses in humans undergoing planned phacoemulsification and IOL insertion (F8, F9, F10). Forty-six patients were dosed with one drop QID 1 day before surgery and one drop 1 hour prior to surgery. Aqueous humor samples were collected, frozen and subsequently analyzed by HPLC to determine the concentration of FQ. All participants were masked throughout the investigation. The results are given in Table 9. In their 46-patient study, Aronowicz et al (F8) showed that moxifloxacin accumulated at levels twice that of gatifloxacin in the aqueous humor: 1.86 ± 0.23 μg/ml for moxifloxacin vs. 0.94 ± 0.15 μg/ml for gatifloxacin; P = 0.001.

Moxifloxacin, Ciprofloxacin, and Ofloxacin (Cataract)

In this study, Solomon et al[14] compared moxifloxacin, ciprofloxacin, and ofloxacin in a double-masked

[F8] Aronowicz JD, Shine W, McCulley JP: Aqueous humor concentrations of fourth-generation fluoroquinolones in humans. *Invest Ophthalmol Vis Sci* 46: E-Abstract 5051, 2005.
[F9] McCulley JP, Surratt G, Shine W: 4th generation fluoroquinolone penetration into aqueous humor in humans. Invest Ophthalmol Vis Sci 45: E-Abstract 4927, 2004.
[F10] McCulley JP, Shine WE: Comparative penetration of 2 fourth-generation fluoroquinolones into the aqueous humor of humans. Presented as Paper PA077 during the Oct 23-26, 2004 Cataract Free Paper Session of the American Academy of Ophthalmology Meeting in New Orleans, LA.

cataract patient study involving 52 cataract patients receiving one of the three antibiotic products four times a day for 3 days prior to surgery and an additional four doses at 15-minute intervals in the hour prior to surgery (16 total doses). Aqueous humor (0.1 ml) was collected during the surgical procedure. The aspirate was immediately frozen at −70°C. Fluoroquinolone concentrations were determined by reverse phase HPLC. The mean aqueous humor drug concentrations are shown in Table 9. Both moxifloxacin (P < 0.001) and gatifloxacin (P < 0.005) penetrated into the aqueous humor significantly better than ciprofloxacin, while moxifloxacin also penetrated into the aqueous better than gatifloxacin (P < 0.05).

Summary of Aqueous Humor Penetration

A summary of the findings from 13 aqueous humor clinical studies is shown in Table 9.

Penetration into Human Vitreous Humor

In the Hariprasad et al study,[7] moxifloxacin concentrations were also determined in the vitreous humors of 20 vitrectomy patients following topical administration of moxifloxacin ophthalmic solution 0.5% for 3 days at 2- or 6-hour intervals.[7] The mean moxifloxacin concentrations were 0.11 and 0.06 μg/ml in the vitreous humor for the 2- and 6-hour regimens, respectively (Table 9). These values were not significantly different (P = 0.08, t-test, 2-tailed homscedastic function). These concentrations did not exceed the MIC90s for these organisms but did exceed the MIC50 values. In contrast, Lott showed that

TABLE 9

Summary of Human Clinical Studies: Penetration of Moxifloxacin, Gatifloxacin, Ciprofloxacin, and Ofloxacin into Human Aqueous Humor

Reference	Patients	Topical Dosage	Fluoroquinolone Aqueous Humor Concentration μg/mL
Moxifloxacin 0.5% (VIGAMOX®)			
Hariprasad 2005[7]	9 Vitrectomy	1 drop every 2 hours for 3 days prior to surgery (43 drops)	2.28 ± 1.23
Hariprasad 2005[7]	10 Vitrectomy	1 drop every 6 hours for 3 days prior to surgery (22 drops)	0.88 ± 0.88
Kim 2005[9]	25 Cataract	1 drop every 10 minutes prior to surgery (4 drops)	1.80 ± 1.21
Solomon 2005[14]	14 Cataract	4 times/day for 3 days and 3 doses 1 hour before surgery (15 drops)	1.31 ±0.46
Aronowicz 2005(F8)	23 Phaco/IOL	4 times/day for day before surgery and 1 hour before surgery (5 drops)	1.86 ± 0.23
Katz 2005[8]	60 Cataract	Regimen 1: 4 drops pre-surgery (4 drops)	1.55 ± 0.86
Katz 2005[8]	60 Cataract	Regimen 2: 4 times/day for day before surgery and 4 drops pre-surgery (8 drops)	1.61 ± 0.71
Gatifloxacin 0.3 % (Zymar®)			
Chu 2004(F13)	25 cataract	4 times/day for 3 days and 1 drop every 15 minutes for 1.5 hours prior to surgery (18 drops)	1.10*
Kim 2005[9]	25 Cataract	1 drop every 10 minutes prior to surgery (4 drops)	0.48 ± 0.34
Price 2005[13]	10 Cataract	4x/day for 2 days before surgery and 1 drop every 10 minutes 1 hour before surgery (14 drops)	1.26 ± 0.55
Solomon 2005[14]	16 Cataract	4 times/day for 3 days and 3 doses 1 hour before surgery (15 drops)	0.63 ± 0.30
Aronowicz 2005(F8)	23 Phaco/IOL	4 times/day for 1 day before surgery and 1 hour before surgery (5 drops)	0.94 ± 0.15
Katz 2005[8]	60 Cataract	Regimen 1: 4 drops pre-surgery (4 drops)	0.74 ± 0.66
Katz 2005[8]	60 Cataract	Regimen 2: 4 times/day for day before surgery and 4 drops pre-surgery (8 drops)	0.91 ± 0.54
Ciprofloxacin 0.3% (Ciloxan®)			
Cekic 1999[2]	18 Vitrectomy	During 6 hours before surgery: 2 drops every 30 minutes for 1st 3 hours and every 60 minutes for next 3 hours (9 drops)	0.44 ± 0.07 SEM
Solomon 2005[14]	22 Cataract	4 times/day for 3 days and 3 doses 1 hour before surgery (15 drops)	0.15 ± 0.11
Donnenfeld 1994[4]	12 Cataract	2 drops 90 minutes preop and 2 drops 30 minutes postop (4 drops)	0.072*
Perry 2004(F14)	14 Cataract	4 times/day for 3 days prior to surgery plus every 15 minutes 1 hour prior to surgery (16 drops)	0.11 ± 0.04
Ofloxacin 0.3% (Ocuflox®)			
Cekic 1998[3]	14 Vitrectomy	During 6 hours before surgery: 2 drops every 30 minutes for 1st 3 hours and every 60 minutes for next 3 hours (9 drops)	1.44 ± 0.24 SEM
Donnenfeld 1994[4]	12 Cataract	2 drops 90 minutes preop and 2 drops 30 minutes postop (4 drops)	0.338
Perry 2004(F14)	15 Cataract	4 times/day for 3 days prior to surgery plus every 15 min 1 hour prior to surgery (16 drops)	0.31 ± 0.62
Levofloxacin 0.5% (Quixin®)			
Yamada 2002[16]	20 Cataract	3 drops every 15 minutes 90 minutes presurgery (18 drops)	1.00 ± 0.48
Perry 2004(F14)	14 Cataract	4 times/day for 3 days prior to surgery plus every 15 minutes 1 hour prior to surgery (16 drops)	0.49 ± 0.79

SEM = standard error of mean.
* SD, SEM, or SE not reported.

the vitreous concentration of orally administered moxifloxacin (two 400-mg doses during 18 hours prior to surgery) produced mean levels in the vitreous of 0.86 μg/ml in nine patients (F11).

Penetration into Human Conjunctivae

A recent, unique pharmacokinetic study by Wagner and associates[13] (F3). Healthy volunteers were administered a single drop of either moxifloxacin 0.5%

TABLE 10

Human Study: Concentration of 5 Fluoroquinolones in Human Conjunctivae Following Topical Dosing

Reference	Mean FQ Conc in Human Conjunctivae [μg/g ± SD]				
	Moxifloxacin	Gatifloxacin	Ciprofloxacin	Levofloxacin	Ofloxacin
Wagner 2005[15] (F16)	18.00 ± 16.4	2.54 ± 2.99	2.65 ± 2.01	2.54[*]	1.26 ± 0.88
Number of patients	15	14	12	12	13

[*] Standard deviation, standard error of the mean, or standard error not reported.

TABLE 11

Summary of Human Clinical Studies: Topical Penetration of Moxifloxacin, Ciprofloxacin and Ofloxacin into Human Vitreous Humor

Reference	Patients	Topical Dosage	Fluoroquinolone Vitreous Humor Concentration μg/mL[*]
Moxifloxacin 0.5% (VIGAMOX®) Hariprasad 2005[7]	10 vitrectomy	1 drop every 2 hours for 3 days prior to surgery (43 drops)	0.11 ± 0.05
Hariprasad 2005[7]	9 vitrectomy	1 drop every 6 hours for 3 days prior to surgery (22 drops)	0.06 ± 0.06
Ciprofloxacin 0.3% (Ciloxan®) Cekic 1999[2]	18 vitrectomy	During 6 hours before surgery: 2 drops every 30 minutes for 1st 3 hours and every 60 minutes for next 3 hours (9 drops)	0.22 ± 0.04 (SE)
Ofloxacin 0.3% (Ocuflox®) Cekic 1998[3]	14 vitrectomy	During 6 hours before surgery: 2 drops every 30 minutes for 1st 3 hours and every 60 minutes for next 3 hours (9 drops)	0.37 ± 0.05 (SE)

SE = standard error.
[*] Mean FQ Conc in μg/mL ± SD unless otherwise indicated.

(VIGAMOX®), ciprofloxacin 0.3% (Ciloxan® Laboratories, Inc., Fort Worth, TX]), gatifloxacin 0.3% (Zymar®), ofloxacin 0.3% (Ocuflox®), or levofloxacin 0.5% (Quixin®) topically. Conjunctival biopsies were taken from the dosed eye (one each from temporal and nasal regions of the inferior cul de sac) 20 minutes post-dose. The biopsy and analytical methods used in this study represent a novel, safe and accurate technique for obtaining conjunctival tissue antibiotic concentrations. The specimens were analyzed by a dual analyte reverse phase HPLC method. Subjects were also followed for 1 week. The mean achievable tissue concentrations obtained in the conjunctivae are given in Table 10. The mean concentration (Cmax) of moxifloxacin in the conjunctiva 20 minutes post dose was approximately 18 μg/g as compared to 2.5 μg/g with gatifloxacin. This concentration for moxifloxacin was six- to 14-fold higher than that achieved for either ciprofloxacin (6.8-fold), gatifloxacin (7.1-fold), ofloxacin (14.6-fold), or levofloxacin (7.7-fold). The conjunctival

levels of moxifloxacin were statistically significantly higher than those of the other four FQs (P < 0.001). There was no statistically significant difference between the conjunctival concentrations of the other four FQs when moxifloxacin was excluded from the analysis (P = 0.3549). The investigators suggest that the enhanced penetration may result from moxifloxacin's high biphasic solubility (both lipophilic and aqueous solubility).

Discussion/Conclusions

Data across multiple in vitro, ex vivo, animal and human clinical studies consistently demonstrate a trend of superior ocular penetration of moxifloxacin compared to other topical fluoroquinolones. This publication includes the major findings and conclusions of previously published or abstracted work as well as original data collected by Alcon.

In vitro and ex vivo studies indicate that this difference is due to the molecular structure of moxifloxacin and specifically its high lipophilicity combined with its high aqueous solubility. Moxifloxacin is unusual among fourth-generation fluoroquinolones in having a bicyclic amine side chain at the C7

[F11] Lott MN, Fuller JJ, Robertson SM, Curtis MA, Dahlin DC, Singh H, Marcus DM: Vitreous penetration of orally-administered moxifloxacin in humans. Invest Ophthalmol Vis Sci 46: E-Abstract 4896, 2005.

position, conferring hydrophilicity along with lipophilicity. In *in vitro/ex vivo* models, moxifloxacin penetrated corneal tissues 3.6-fold better than gatifloxacin and two-fold faster. VIGAMOX® maintained corneal integrity better than Zymar®. Moxifloxacin was the most lipophilic fluoroquinolone of the seven fluoroquinolones tested and showed the greatest aqueous solubility. The presence of the bulky bicyclo amine substituent inhibits the bacterial active efflux pump (F12). As a result, moxifloxacin has sufficient lipophilicity to readily cross the corneal epithelium while simultaneously it has high aqueous solubility and potency at physiological pH. For example, moxifloxacin has an aqueous solubility at pH 7 of up to at least 3.0%. In contrast, the maximum solubility of ciprofloxacin at this pH is about 0.01%. The high solubility of moxifloxacin results in higher tear film concentrations providing a driving force (concentration gradient) for corneal uptake.

Various *in vivo* animal and human clinical studies support the fact that moxifloxacin penetrates ocular tissues better than other fluoroquinolones when instilled topically. In the single-dose animal studies, the instillation of a single drop topically of a 0.3% solution of moxifloxacin achieved maximum levels in the rabbit cornea, aqueous humor and iris-ciliary bodies of 12.5, 1.8, and 13.5 μg/ml or g, respectively. Concentrations of moxifloxacin were typically two-fold higher than the corresponding values for ofloxacin and remained two-fold higher throughout the study. Moxifloxacin concentration in the cornea was 0.25 μg/g at 48 hours, or about four-fold above the MIC for methicillin-susceptible *Staphylococcus aureus*. In contrast, ofloxacin corneal concentrations were below its MIC threshold by 8 hours. The mean moxifloxacin tear film concentration at the initial 10-minute time-point was 366 μg/ml and remained at or above 1 μg/ml for up to at least 6 hours post-dose. Plasma concentrations of both drugs were low (0.01 μg/ml or less) and declined rapidly. Moxifloxacin exhibited a better penetration profile than ofloxacin which penetrates better than ciprofloxacin. Maximal moxifloxacin levels in ocular tissues were typically two-fold higher than ofloxacin levels and generally remained higher than those of ofloxacin over time. In the cornea, moxifloxacin levels were about four-fold higher than the MIC$_{90}$s for MRSA strains even at 48 hours post-dose, whereas ofloxacin levels fell below its MIC$_{90}$ by 8 hours. After topical dosing, tear film concentrations of moxifloxacin remained higher (seven- to 10-fold) than ofloxacin over time. Ofloxacin levels at 90 minutes were below the quantitation limits, but moxifloxacin remained at or

above 1 μg/ml up to the last time point at 6 hours. Systemic exposure was low following topical ocular administration. Based on plasma levels determined in toxicology and clinical studies at well tolerated doses, 0.3% moxifloxacin solution has a wide margin of safety.

In multidose animal studies by Robertson, moxifloxacin exhibited a better penetration profile than gatifloxacin or ofloxacin (F1). Maximal moxifloxacin levels in the ocular tissues were typically three- to six-fold higher than ofloxacin or gatifloxacin. Upon topical instillation, moxifloxacin achieved levels of 0.2 to 0.4 μg/ml in the vitreous humor in animals or 0.06 to 0.11μg/ml in humans. These levels were considerably lower than those obtained in the aqueous in these studies, but exceeded MIC$_{90}$ for moxifloxacin against *Staphylococcus epidermidis*, *S. aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae* and other gram-negative bacteria. Although MICs are related to the efficacy in the treatment or prevention of infection, they are not always predictive. For example, even though moxifloxacin has a higher MIC against *Pseudomonas aeruginosa*, *in vivo* infections studies at Wilmer Eye Institute have shown that topical moxifloxacin 0.5% was as effective as ciprofloxacin 0.3% in treating infections with this particularly troublesome organism.[1]

These topical penetration studies in animals fairly well predicted what happens in humans. The rabbit data clearly give an edge to moxifloxacin as the fluoroquinolone that penetrates the best into the aqueous after topical treatment. This is also seen in the human clinical trials summarized in Tables 10 and 11. Although there are some low accumulations of fluoroquinolones in the vitreous humor via the topical route, the concentrations are at or near the MICs of susceptible strains of pathogens, but the levels are not high enough for therapeutic considerations (Table 11).[2,3,7] Nevertheless, there is extensive penetration of moxifloxacin into the human conjunctiva after topical instillation (Table 10)[15] (F3). This is not surprising, since Gipson demonstrated the similarity of the cell-to-cell and cell-to-substrate junctions between cornea and conjunctiva.[6]

The concentration of fluoroquinolone present in commercial products certainly influences potency relative to potency of products. Upon topical administration of moxifloxacin ophthalmic solution 0.5%, moxifloxacin penetrates ocular tissues more extensively than

---

F12 Avelox® Package Insert, Bayer Pharmaceuticals Corporation, 2004.

F13 Chu Y: Penetration of gatifloxacin ophthalmic solution 0.3% into aqueous humor of patients undergoing cataract surgery. Invest Ophthalmol Vis Sci 45: E-Abstract 4007, 2004.

F14 Perry HD, Donnenfeld E, Bloom A, Snyder R, Levine J, Mychajlyszyn J, Greenman H, Solomon R: Aqueous humor concentrations of topically applied fluoroquinolones. Invest Ophthalmol Vis Sci 45: E-Abstract 4932, 2004.

other topical fluoroquinolones, giving substantial and prolonged aqueous humor, corneal, iris-ciliary body, and tear concentrations well above the MICs for common ocular pathogens.[10] The higher concentration of moxifloxacin in the commercial preparation (i.e., VIGAMOX® with moxifloxacin at 0.5%) compared to 0.3% gatifloxacin in Zymar®, 0.3% ciprofloxacin in Ciloxan®, or 0.3% ofloxacin in Ocufloxin also enhances its penetration.

Method of Literature Search

We performed an international literature search for this article based on MEDLINE database searches from 1990 to 2005, using varying combinations of the search terms: *moxifloxacin, fluoroquinolones, ocular penetration, aqueous humor*. All relevant journal articles and/or abstracts were selected for review. English abstracts were used for non-English papers. Recent papers presented at ARVO were also included for completeness.

References

1. Aliprandis E, Ciralsky J, Lai H, et al: Comparative efficacy of topical moxifloxacin versus ciprofloxacin and vancomycin in the treatment of P. aeruginosa and ciprofloxacin-resistant MRSA keratitis in rabbits. Cornea 24:201–5, 2005

2. Cekic O, Batman C, Yasar U, et al: Human aqueous and vitreous humour levels of ciprofloxacin following oral and topical administration. Eye 13:555–8, 1999

3. Cekic O, Batman C, Yasar U, et al: Penetration of ofloxacin in human aqueous and vitreous humors following oral and topical administration. Retina 18:521–5, 1998

4. Donnenfeld ED, Schrier A, Perry HD, et al: Penetration of topically applied ciprofloxacin, norfloxacin and ofloxacin into the aqueous humor. Ophthalmology 101:902–5, 1994

5. Fukada M, Sasaki K: In vitro topically applied fluoroquinolone penetration into the anterior chamber. *Nippon Ganka Gakkai Sasshi* 99:532–6, 1995

6. Gipson IK, Joyce NC: Anatomy and cell biology of the cornea, superficial limbus and conjunctiva. in Albert DM, Jacobiec FA (eds): Principles and Practices of Ophthalmology, 2nd ed. W.B. Saunders Co, Philadelphia, PA, USA 2000, ed 2, chap 56, pp 625–6

7. Hariprasad SM, Blinder KJ, Shah GK, et al: Penetration pharmacokinetics of topically administered 0.5% moxifloxacin ophthalmic solution in human aqueous and vitreous. Arch Ophthalmol 123:39–44, 2005

8. Katz HR, Masket S, Lane SS, et al: Absorption of topical moxifloxacin ophthalmic solution into human aqueous humor. Cornea (in press)

9. Kim DH, Stark WJ, O'Brien TP, Dick JD: Aqueous penetration and biological activity of moxifloxacin 0.5% ophthalmic solution and gatifloxacin 0.3% solution in cataract surgery patients Ophthalmology, in press.

10. Kowalski RP, Dhaliwal DK, Karenchak LM, et al: Gatifloxacin and moxifloxacin: an *in vitro* susceptibility comparison to levofloxacin, ciprofloxacin, and ofloxacin using bacterial keratitis isolates. Am J Ophthalmol 136:500–5, 2003

11. Levine JM, Noecker RJ, Lane LC, et al: Comparative penetration of moxifloxacin and gatifloxacin in rabbit aqueous humor after topical dosing. J Cataract Refract Surg 30:2177–82, 2004

12. Mather R, Stewart JM, Prabriputaloong T, et al: The effect of cataract surgery on ocular levels of topical moxifloxacin. Am J Ophthalmol 138:554–9, 2004

13. Price MO, Quillin C, Price FW: Effect of gatifloxacin ophthalmic solution 0.3% on human corneal endothelial cell density and aqueous humor gatifloxacin concentration. Cur Eye Res 30:563–7, 2005.

14. Solomon R, Donnenfeld ED, Perry HD, et al: Penetration of topically applied gatifloxacin 0.3%, moxifloxacin 0.5%, and ciprofloxacin 0.3% into the aqueous humor. Ophthalmology 112:466–9, 2005

15. Wagner RS, Abelson MB, Shapiro A, Torkildsen G: Evaluation of moxifloxacin, ciprofloxacin, gatifloxacin, ofloxacin, and levofloxacin concentrations in human conjunctival tissue. Arch Opthalmol 123:1282–3, 2005

16. Yamada M, Mochizuki H, Yamada K, et al: Aqueous humor levels of topically applied levofloxacin in human eyes. Curr Eye Res 24:403–6, 2002

ELSEVIER

Safety of Moxifloxacin as Shown in Animal and *In Vitro* Studies

David H. McGee, PhD, William F. Holt, PhD, Philip R. Kastner, PhD, and Rebecca L. Rice, DVM, PhD

Alcon Research, Ltd., Fort Worth, Texas, USA

Abstract. Topical treatment of ocular bacterial infection is practiced widely, and the choice of the antibacterial agent depends on the nature of the infection, including the susceptibility of the organism, the tissue affected, and the safety profile of the agent. Moxifloxacin is a fourth-generation fluoroquinolone approved for ophthalmic use as moxifloxacin ophthalmic solution 0.5% (VIGAMOX®, Alcon, Fort Worth, TX). Moxifloxacin ophthalmic solution 0.5% is self-preserved at a near-neutral pH of 6.8. In treating ocular infection, the three important aspects of therapeutic control are potency, penetration of the drug to the target site, and safety of the drug and the drug product. Moxifloxacin ophthalmic solution 0.5% provides antibacterial potency and high penetration of target ocular tissues. The ocular and systemic safety profile of moxifloxacin compares favorably with those of other fluoroquinolone antimicrobial agents, with a low risk of recognized quinolone-related toxicity. *In vitro* studies of fluoroquinolones with human or rabbit corneal epithelial cells or keratocytes suggest that moxifloxacin is similar in cytotoxicity potential to other drugs of this family. Specialized *in vivo* corneal wound-healing studies draw little distinction between moxifloxacin-treated eyes and those treated with other fluoroquinolones. Repeated-dose topical ocular studies in rabbits and monkeys, with high concentrations (up to 3%) of moxifloxacin and at treatment durations and regimens well in excess of label-prescribed use, demonstrated a high safety margin for ocular and extraocular tissues. Cornea, the tissue with highest exposure, was found to be unaffected by these high exposures, with slit-lamp biomicroscopy, corneal thickness measurement, intraocular pressure, and specular microscopy of the corneal endothelium (monkeys only) during in-life evaluation, and histologic evaluation showing no effects, as compared with controls. Moxifloxacin ophthalmic solution 0.5% affords superior efficacy and ocular tissue penetration, with a favorable safety profile. (Surv Ophthalmol 50:S46–S54, 2005. © 2005 Elsevier Inc. All rights reserved.)

Key words. fluoroquinolone · moxifloxacin · nonclinical safety · safety pharmacology · toxicity · VIGAMOX®

Drug safety is assessed through a rigorous nonclinical testing program in drug development. It is estimated that five new medicinal entities advance to testing in humans out of 5,000 candidate drugs, and only one of these five may ultimately gain regulatory approval. Nonclinical assessment includes efficacy and safety pharmacology, pharmacokinetics/metabolism, and toxicology studies. Fluoroquinolones are generally considered safe and well tolerated, as compared with other commonly prescribed antimicrobials.[6]

TABLE 1

Product Synopsis: Ophthalmic Fluoroquinolones[*]

	Ciloxan®	Ocuflox®	Quixin®	VIGAMOX®	Zymar®
Drug	Ciprofloxacin 0.3%	Ofloxacin 0.3%	Levofloxacin 0.5%	Moxifloxacin 0.5%	Gatifloxacin 0.3%
Benzalkonium chloride	0.006%	0.005%	0.005%	—	0.005%
Edetate disodium	0.05%	—	—	—	+
Sodium chloride	+	+	+	+	+
Sodium acetate	+	—	—	—	—
Acetic acid	+	—	—	—	—
Boric acid	—	—	—	+	—
Mannitol	4.6%	—	—	—	—
pH	4.5	6.4	6.5	6.8	6
Osmolality	300	300	300	290	260–330
Dose regimen initial	Days 1 and 2: 1–2 drops every 2 hours	Days 1 and 2: 1–2 drops every 2–4 hours	Days 1 and 2: 1–2 drops every 2 hours	1 drop t.i.d., (38-µL drop)	Days 1 and 2: 1 drop every 2 hours
Follow-up	Days 3–7: 1–2 drops every 4 hours	Days 3–7: 1–2 drops 4 times a day	Days 3–7: 1–2 drops 4 times a day		Days 3–7: 1–2 drops 4 times a day
Plasma C_{max} (ng/mL)	2.5	1.9	2.25	2.7	<5

+ = present in the product, concentration not specified; − = not present in the product; C_{max} = peak concentration achieved in the tissue.

[*] PDR Electronic Library Online: Thomson MICROMEDEX, http://www.thomsonhc.com/pdrel/librarian/ND_PR/Pdr, 2004.

Structural refinements have improved the safety and efficacy profiles of the new fourth-generation fluoroquinolones (i.e., moxifloxacin, gatifloxacin, and trovafloxacin). The approval of moxifloxacin ophthalmic solution 0.5% (VIGAMOX®, Alcon, Fort Worth, TX) and gatifloxacin ophthalmic solution 0.3% (Zymar®, Allergan, Irvine, CA) by the US Food and Drug Administration in 2003 added two powerful fourth-generation fluoroquinolones to the ophthalmologist's armamentarium. These new drugs offer high potency against ocular pathogens, with spectrum advantages over the earlier-generation ophthalmic fluoroquinolones ciprofloxacin ophthalmic solution 0.3% (Ciloxan®, Alcon), ofloxacin ophthalmic solution 0.3% (Ocuflox®, Allergan), and levofloxacin ophthalmic solution 0.5% (Quixin, Vistakon, Jacksonville, FL). Moxifloxacin ophthalmic solution is an iso-osmotic solution containing 0.5% moxifloxacin, boric acid, and sodium chloride, with an approximate pH of 6.8. Moxifloxacin ophthalmic solution 0.5% is self-preserved, with no designated preservative added (F1). Gatifloxacin ophthalmic solution 0.3% contains gatifloxacin at a concentration of 0.3%, with benzalkonium chloride 0.005% as a preservative, ethylenediaminetetra-acetic acid, and sodium chloride. The solution is approximately pH 6 (no pH buffer added) and iso-osmotic. A synopsis of product characteristics is presented in

Table 1. Moxifloxacin ophthalmic solution 0.5% and gatifloxacin ophthalmic solution 0.3% combine the advantages of high local concentrations of antibiotic at the site of action with low systemic exposure levels after therapeutic use. Plasma drug levels associated with topical dosing are about 1,000-fold below those encountered with systemic use of fluoroquinolones. Therefore, these factors suggest that the topical application of these antibiotics do not contribute to systemic toxicity or the development of antimicrobial resistance.

Nonclinical Studies

Nonclinical studies conducted to assess the safety of moxifloxacin summarized here include safety pharmacology studies on major physiological systems and drug interaction potential, and toxicology studies to identify target organs and estimate safety margins.

SAFETY PHARMACOLOGY STUDIES

Safety pharmacology studies profiled effects of moxifloxacin on major organ systems, with particular attention to those known to be most affected—for example, the central nervous system, gastrointestinal tract, and cardiovascular system. Selected safety pharmacology study results are summarized in Table 2. The safety margins determined in these studies ranged from 400- to 7,000-fold, providing strong support that ophthalmic use of moxifloxacin is unlikely to result in significant adverse effects in humans.

F1 Schlech BA, Sutton S, Rosenthal RA, et al: Antimicrobial preservative effectiveness of VIGAMOX®™ (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4913, 2004.

TABLE 2

Safety Pharmacology Studies for Moxifloxacin

System/Study Type	Species/Route	No Effect Dose[*]	Safety Margin[†]
Central nervous system			
Analgesia	Mouse/oral	100 mg/kg	>4,300 ×
Sensorimotor	Mouse/oral	30 mg/kg	>1,300 ×
Convulsant	Mouse/oral	30 mg/kg	>1,300 ×
Psychomotor	Rat/oral	30 mg/kg	>1,300 ×
Gastrointestinal			
Gastric acid secretion	Rat/intraduodenal	100 mg/kg	>4,300 ×
Indomethacin-induced erosin	Rat/oral	100 mg/kg	>4,300 ×
Acetylcholine, serotonin, histamine, $BaCl_2$-induced spasm	Guinea pig ileum/ *in vitro*	10 µg/mL	>3,000 ×
Cardiovascular			
HERG channel (repolarization)	CHO cells/ *in vitro*	30 µM	>4,000 ×
Papillary muscle	Guinea pig/ *in vitro*	50 µM	>7,000 ×
Isolated cardiac myocyte assay	Guinea pig/ *in vitro*	50 µM	>7,000 ×
Torsades-de-pointes model	Rabbit/IV	120 mg/kg in 1 hour	>5,000 ×
ECG (QT/QTc interval)	Dog/IV	10 mg/kg	>430 ×
	Monkey/intraduodenal	100 mg/kg	>4,300 ×
Renal			
Urine volume and electrolyte excretion	Rat/oral	100 mg/kg	>4,300 ×
	Rat/IV	10 mg/kg (small change in K⁺ at 30 mg/kg)	>430 ×
Respiratory			
Pulmonary resistance, lung compliance, and respiration rate	Guinea pig/oral	100 mg/kg	>4,300 ×
HR, BP, respiration rate, pulmonary function	Guinea pig/IV	10 mg/kg	>430 ×
Other			
Blood glucose levels	Rat/IV	30 mg/kg	>1,300 ×

BP = blood pressure; CHO = Chinese hamster ovary; ECG = electrocardiogram; HERG = human ether-a-go-go-related gene; HR = heart rate; IV = intravenous.

[*] That elicited no statistically or biologically significant pharmacologic response.

[†] Ratio of no effect dose (mg/kg) to clinical dose (0.023 mg/kg), (based on 100% absorption of 1 drop 0.5% moxifloxacin solution, both eyes, three times a day, to a 50 kg patient) for the *in vivo* studies, or mean clinical C_{max} (2.7 ng/mL) for *in vitro* studies using exposure by concentration (µM or µg/mL).

Moxifloxacin produced no overt central nervous system effects in rats or mice at a 30-mg/kg dose in a battery of central nervous system screening studies for analgesia, convulsant, sensorimotor, and psychomotor activity. Sedation was observed in rodents at the highest oral dose tested (100 mg/kg), with no effect observed at 30 mg/kg.

Fluoroquinolone antibiotics are recognized to produce prolongation of the QTc interval of the electrocardiogram, and *in vitro* and *in vivo* studies were conducted to assess this potential for moxifloxacin. These studies were performed by Alcon and Bayer in support of the US New Drug Application of VIGA-MOX® and included guinea pig papillary muscle action potential, patch clamp studies, human ether-a-go-go–related gene (HERG) channel studies, a special rabbit model of torsades de pointes, and electrocardiogram assessments in dogs and monkeys. Intravenously infused moxifloxacin (10 mg/kg)

produced no electrocardiogram changes in dogs, and no significant human ether-a-go-go–related gene channel inhibition was observed with high concentrations of moxifloxacin.[11] Safety pharmacology studies thus demonstrated high therapeutic indices for physiological systems of concern with fluoroquinolone use, emphasizing the benefit of local antibacterial efficacy with minimal systemic risk.

TOXICOLOGY STUDIES

Toxicology studies were conducted to establish the pharmacotoxicological profile of moxifloxacin, to assure safe use in clinical trials and to support drug safety for worldwide marketing applications. Systemic toxicity studies conducted by Bayer established the safety profile for moxifloxacin in support of oral and intravenous drug products (Avelox®, Bayer). Additional studies were conducted by Alcon to assess safety with topical ophthalmic use. Further investigations

relating to special-use situations have been reported in published literature. The systemic and general toxicology profile of moxifloxacin has been described.[19] Principal target organs were identified as the liver, heart, central nervous system, and bone marrow. "No effect" doses were well in excess of therapeutic doses for systemic use and generally ranged from 30 to 100 mg/kg/day. Toxicity specifically associated with fluoroquinolones (e.g., phototoxicity, arthropathy, electrocardiogram changes) was observed only at relatively high doses (>30 mg/kg). No effects were observed on fertility, reproduction, or embryo-fetal development at doses of 30 mg/kg or below. Genotoxic effects were seen in some assays, but these effects are common to all fluoroquinolones and related to the antimicrobial mechanism of action of the drug. An accelerated carcinogenicity bioassay suggested no tumorigenic activity.

OCULAR STUDIES

In Vivo Studies

Drug exposure by topical ophthalmic use is low compared with systemic doses. The plasma peak concentration achieved in the tissue for systemic therapy is 4–5 µg/mL, whereas the average highest plasma concentrations in subjects receiving topical moxifloxacin ophthalmic solution 0.5% is reported to be 2.7 ng/mL—a difference of more than 1,000-fold.

An eyedrop delivers an initial concentration of drug equivalent to that of the product. It is estimated that most of an administered eyedrop is lost to drainage in the first 15 to 30 seconds after instillation.[17] The tear turnover rate is approximately 16% per minute, so that nearly all of the drug is expected to disappear within 10 minutes after dosing. Maurice and Mishima estimated that the instilled drug is diluted in the tear fluid, and after drainage of excess fluid, the drug concentration averages about one third of the original drop.[14] Robertson et al showed that the concentration of moxifloxacin in tears following a single dose of moxifloxacin ophthalmic solution 0.5% in rabbits was approximately 400 µg/mL initially, but dropped to 1–10 µg/mL by about 30 minutes post-instillation (F2). Thus, exposure of ocular tissue, including corneal epithelium, to the drug at the product concentration will be very short-lived.

Nonclinical studies to assess the safety of moxifloxacin ophthalmic solution were conducted in rabbits and monkeys by the clinical route of administration. In these studies, standard animal models were used

with high exposure to the drug (by increased drug concentration, dose regimen, and/or treatment duration) and incorporated thorough ophthalmologic and systemic assessments. These measures help establish safety margins for both systemic exposure and local effects on the eye and ocular adnexa.

Two studies employed different rabbit varieties, the albino New Zealand White and a pigmented NZ White × NZ Red cross (F3). A pigmented variety was desired because fluoroquinolones possess high binding affinity to melanin. In both studies, animals were dosed with two drops, to the right eye, four times a day, for approximately 1 month. Treatments were 0% (vehicle), 0.5%, 1.0%, and 3.0% moxifloxacin solutions. Ocular evaluations, including slit-lamp biomicroscopy, corneal thickness measurements, indirect ophthalmoscopy, and microscopic evaluation, revealed no significant effects. Systemic parameters, such as body weight, general health, clinical laboratory tests, and histopathology, also showed no significant treatment-related effects.

A subchronic ophthalmic safety study in cynomolgus monkeys employed a dosing regimen of two drops to the right eye, six times a day for 16 days, followed by t.i.d. dosing for the remainder of the 3-month study (F4). Treatments were 0% (vehicle), 0.5%, 1.0%, and 3.0% moxifloxacin solutions. Ocular and systemic evaluations included those described for the rabbit studies, as well as intraocular pressure and specular microscopy of the corneal endothelium. All ophthalmic parameters were comparable in the untreated eyes, vehicle controls, and moxifloxacin ophthalmic solution–treated eyes. Corneal thickness, a sensitive indicator of corneal health, was not affected by administration of moxifloxacin ophthalmic solution, even at the high concentrations and extreme regimen employed. Mean corneal endothelial cell density and cell area were comparable in vehicle control and moxifloxacin-treated eyes, further establishing corneal safety. No significant findings were observed for any systemic parameters.

The effects of topical administration of moxifloxacin and gatifloxacin ophthalmic solutions have been investigated with alternative treatment regimens. These studies employed normal healthy corneas. Herrygers et al reported that New Zealand White rabbits dosed with moxifloxacin ophthalmic solution 0.5% under either a keratitis regimen (one drop

F2 Robertson SM, Sanders M, Jasheway D, et al: Penetration and distribution of moxifloxacin and ofloxacin into ocular tissues and plasma following topical ocular administration to pigmented rabbits (abstract). Invest Ophthalmol Vis Sci 44(Suppl):1454, 2003.

F3 McGee DH, Heaton J, Hackett R, et al: Toxicity of moxifloxacin ophthalmic solution 0.5% in the rabbit (abstract). Invest Ophthalmol Vis Sci 44(Suppl):4458, 2003.

F4 Bergamini MV, Heaton J, McGee D, et al: A three-month topical ocular toxicity study of moxifloxacin ophthalmic solutions in cynomolgus monkeys (abstract). Invest Ophthalmol Vis Sci 44(Suppl):4457, 2003.

every 5 minutes for 15 minutes, followed by one drop every 15 minutes for 4 hours) or a post–cataract surgery prophylaxis regimen (four times daily for 10 days) showed no significant corneal epithelial damage by scanning electron microscopic evaluation by either regimen.[9] Similar results were seen with gatifloxacin ophthalmic solution 0.3% and vehicle in this study, and it was concluded that both products were well tolerated by the ocular surface and considered safe and nontoxic. The effects of ophthalmic solutions of moxifloxacin, ciprofloxacin, levofloxacin, and ofloxacin on corneal epithelium and stroma were assessed by confocal microscopy in rabbits.[12] All products except moxifloxacin ophthalmic solution 0.5% contain 0.005% or 0.006% benzalkonium chloride. Tears Naturale Free (Alcon) was used as a control. After 6 days of treatment, corneal epithelial thickness was decreased from baseline for all drug-treated groups except moxifloxacin ophthalmic solution 0.5% and Tears Naturale Free, whereas corneal stromal thickness was similar to baseline.

Corneal Wound Healing Studies

Some studies have suggested effects of fluoroquinolones on corneal wound healing. Heavy dosing of de-epithelialized rabbit corneas with ofloxacin solution inhibited epithelial cell regrowth and resulted in keratocyte loss, as compared with both untreated and intact corneas.[15] Levofloxacin delayed re-epithelialization and resulted in some keratocyte loss, stromal swelling, and disorganization when dosed to de-epithelialized corneas of rabbits at high (3% and 6%) concentrations.[2] Delayed re-epithelialization, increased corneal thickness, and haze were observed in monkeys with the same treatment. These results suggest that ofloxacin and levofloxacin, in high doses, may result in some slight effects on corneal recovery.

Nonclinical studies have also been conducted to assess the safety of fourth-generation fluoroquinolones in the wounded cornea. Some reports have suggested that moxifloxacin ophthalmic solution 0.5% affects corneal wound healing. The healing of linear incisions and anterior keratectomy wounds were reported to be slightly slower in rabbit corneas treated with 0.5% moxifloxacin solution, as compared with 0.3% gatifloxacin solution (F7, F6).

However, an increasing number of nonclinical studies have shown no differences in corneal wound healing between moxifloxacin ophthalmic solution 0.5% and gatifloxacin ophthalmic solution 0.3%.

McCartney et al reported no differences were observed in slit-lamp biomicroscopy scores, histology, or electron microscopy between rabbit corneas treated with moxifloxacin ophthalmic solution 0.5% (one drop t.i.d. for 7 days) and those treated with gatifloxacin ophthalmic solution 0.3% (one drop four times a day for 7 days) after penetrating linear incisions (F7).

Sorour et al compared corneal healing with treatment of moxifloxacin, gatifloxacin, or levofloxacin (one drop four times a day; concentrations not stated in abstract) after photorefractive keratectomy in chicken eyes (F8). Chickens were chosen as an experimental model because the chicken eye possesses a prominent Bowman's layer and its cornea is histologically similar to the human cornea (F9). Wound sizes over time were comparable in the balanced salt solution control (56.69 hours), moxifloxacin (56.73 hours), and gatifloxacin (56.40 hours) and greater than with levofloxacin (65.64 hours). The overall healing rate over time did not statistically differ between treatment groups. Wound size and percent healing were statistically significantly less for levofloxacin compared with other groups at 60 and 66 hours postoperatively, and the fourth-generation fluoroquinolones appeared slightly less toxic on the epithelium than levofloxacin.

The healing rate was slightly higher for anterior keratectomy wounds in New Zealand White rabbits treated with one drop four times a day for 4 days with moxifloxacin ophthalmic solution 0.5% (87±8%) relative to gatifloxacin ophthalmic solution 0.3% (77±10%), with no significant differences in collagen IV expression (F10). Similar results were obtained in the corneas of pigmented rabbits, where

---

[F5] Schmidt L, Beuerman R: Comparison of gatifloxacin and moxifloxacin in healing of a linear incision in the rabbit cornea (abstract). Invest Ophthalmol Vis Sci 45(Suppl):1427, 2004.

[F6] Gao J, Siemasko K, Vu C, et al: Effect of 4th generation fluoroquinolone on rabbit corneal wound healing (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4889, 2004.

[F7] McCartney MD, Rice RL, Hackett RB, et al: Comparison of wound healing in pigmented rabbits receiving moxifloxacin 0.5% or gatifloxacin 0.3% ophthalmic solutions following penetrating corneal incision. Cornea. Submitted for publication.

[F8] Sorour H, Yee S, Chuang A, et al: Epithelial healing of the topical levofloxacin, gatifloxacin and moxifloxacin after photorefractive keratectomy in chickens and relative toxicity of topical levofloxacin, gatifloxacin, moxifloxacin and on ofloxacin on human corneal epithelial cell culture. Cornea. Submitted for publication.

[F9] Fowler WC: An animal model for LASIK flap research: the white leghorn chicken (abstract). Invest Ophthalmol Vis Sci 41(Suppl):S459, 2000.

[F10] Williams KK, Munger RJ, Shepard AR, et al: Corneal wound healing in New Zealand White rabbits following anterior keratectomy and treatment with moxifloxacin 0.5% ophthalmic solution or gatifloxacin 0:3% ophthalmic solution. Cornea. Submitted for publication.

wound healing percentages were 90±8% and 81±14% for moxifloxacin ophthalmic solution 0.5%–treated and gatifloxacin ophthalmic solution 0.3%–treated eyes, respectively (F11).

Most *in vivo* studies in animal models suggest that there are no significant adverse effects of the fourth-generation fluoroquinolones, moxifloxacin and gatifloxacin. Indeed, the fourth-generation fluoroquinolones may offer advantages over some older products such as ofloxacin and levofloxacin.

In Vitro Studies

Cutarelli et al compared antimicrobial activity and *in vitro* corneal epithelial toxicity of five fluoroquinolones, two aminoglycosides, and cefazolin, and established that fluoroquinolones were generally more potent against ocular pathogens and less toxic to the corneal epithelial cells.[9] Reports from *in vitro* studies have suggested variable results in relative cytotoxicity when comparing multiple fluoroquinolones. Recently, Yee et al compared the effects of moxifloxacin and gatifloxacin with those of levofloxacin and ofloxacin ophthalmic solutions on human corneal epithelial cells *in vitro* (F12). Cultures were exposed to the marketed products for either 5 or 15 minutes, after which relative numbers of live/dead cells were evaluated. Moxifloxacin was least cytotoxic (83.6%), with gatifloxacin (88.2%), levofloxacin (89.5%), and ofloxacin (90.9%) similar to one another, showing somewhat greater cytotoxicity, compared with moxifloxacin or the controls (Systane® [Alcon], 70.2%, and culture control, 70.9%). Matsumoto et al suggested that moxifloxacin inhibits *in vitro* corneal wound healing more than gatifloxacin, levofloxacin, and ofloxacin, with the order of inhibition ofloxacin = levofloxacin < gatifloxacin < moxifloxacin << ciprofloxacin when tested at 0.6 mM (F13). In a similar study, Matsumoto et al reported ofloxacin inhibited *in vitro* corneal wound healing in the same model.[13] Gatifloxacin, ciprofloxacin, and moxifloxacin, at concentrations of 1 μg/mL or more, were all cytotoxic *in vitro* to human corneal endothelial cells

and keratocytes (F14). Most of these studies used antibiotic solutions and not the commercial topical products. The presence of benzalkonium chloride in products, like Zymar, would result in a markedly different outcome in these cytotoxicity studies. *In vitro* assays may be useful in assessing comparative effects of drugs, or products, but may have limited relevance to clinical use.

The ocular surface is exposed to all components of the ophthalmic drug product, including excipients, and these may affect the cornea and conjunctiva. The antimicrobial preservative in these preparations has a great influence on ocular safety. All approved ophthalmic fluoroquinolone solutions are preserved with benzalkonium chloride, except moxifloxacin ophthalmic solution 0.5% and, recently, levofloxacin ophthalmic solution 1.5% (Iquix®, Vistakon), which have no designated preservative added. The safety of fluoroquinolones with respect to the cornea is of utmost importance, and the toxicological first principle that "the dose makes the poison" emphasizes the importance of safety to the tissues exposed to the highest concentration. Moxifloxacin ophthalmic solution achieves superior concentrations in corneal tissue and aqueous humor when administered topically, as compared with other fluoroquinolones, including gatifloxacin (F15).[16] This suggests the potential for a longer duration of drug concentrations above the MIC (Minimum Inhibitory Concentration) for infecting organisms (F16, F17). Rusinko et al reported that drug permeability and penetration correlate with lipophilicity and aqueous solubility for a series of seven fluoroquinolones (including moxifloxacin, gatifloxacin, and ofloxacin) (F18) and Owen et al demonstrated the superior permeability and penetration characteristics of moxifloxacin (F19). Corneal penetration of the drug was

---

[F11] Williams KK, McCartney MD, Rice RL, et al: The effects of moxifloxacin 0.5% ophthalmic solution or gatifloxacin 0.3% ophthalmic solution treatment on corneal wound healing in pigmented rabbits following anterior keratectomy. Cornea. Submitted for publication.

[F12] Yee RW, Sorour HM, Yee SB, et al: Comparison of relative toxicity of four ophthalmic antibiotics using the human cornea epithelial cell culture system (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4939, 2004.

[F13] Matsumoto S, Way W, Carlo K, Short B: Comparative toxicity of fluoroquinolones antibiotics on corneal cells *in vitro* (abstract). Am College Toxicol:17, 2003.

[F14] Skelnik DL, Clark LA, Bezwada P: Effect of drug concentration and exposure time of levofloxacin, ofloxacin, ciprofloxacin, gatifloxacin and moxifloxacin on human corneal endothelial cells and keratocytes (abstract). Invest Ophthalmol Vis Sci 44(Suppl):4739, 2003.

[F15] Robertson SM, Sanders M, Jasheway D, et al: Absorption and distribution of moxifloxacin, ofloxacin and gatifloxacin into ocular tissues and plasma following topical ocular administration to pigmented rabbits (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4906, 2004.

[F16] Levine J, Noecker R, Herrygers L, Clark T: Aqueous levels of moxifloxacin and gatifloxacin following different pre- and post-operative topical dosing protocols (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4911, 2004.

[F17] Mather R, Stewart JM, Prabriputaloong T, et al: Corneal concentrations of moxifloxacin following topical administration in a rabbit model (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4989, 2004.

much greater and more rapid for moxifloxacin ophthalmic solution 0.5% (91×10^{-7} cm/second; lag time, 49 minutes) than for gatifloxacin ophthalmic solution 0.3% (25×10^{-7} cm/second; lag time, 99 minutes), whereas corneal permeability to carboxyfluorescein was much greater after gatifloxacin ophthalmic solution 0.3% exposure (3.6 pM/mL/minute) than after moxifloxacin ophthalmic solution 0.5% (2.1 pM/mL/minute). Carboxyfluorescein does not normally cross the cornea but is an indicator of corneal permeability through epithelial cell junction disruption, as it readily passes through the disrupted epithelium. Thus, the superior penetration profile of moxifloxacin, nearly four times more, is attributable to the inherent physicochemical properties of the drug and not to disruption of tight junctions in the corneal epithelium. The carboxyfluorescein findings with gatifloxacin ophthalmic solution 0.3% are consistent with those of benzalkonium chloride–containing formulations, where the permeability is increased in a concentration-dependent manner. Benzalkonium chloride concentrations as low as 0.005% applied to the rabbit eye (15 times at 5-minute intervals) caused superficial epithelial swelling and desquamation.[10]

Clinical Relevance of Animal and *In Vitro* Findings

In vitro and animal studies in intact corneas consistently show that topical ophthalmic fluoroquinolones are safe even at very high concentrations and treatment regimens, and this seems consistent with clinical experience. Nguyen et al found no significant effects on conjunctival injection, chemosis, stinging/burning, lid thickness, ocular surface sensation, or pupil size after a single drop of moxifloxacin ophthalmic solution 0.5% to one eye and gatifloxacin ophthalmic solution 0.3% to the fellow eye of 10 normal subjects (**F20**). This was confirmed in a study of human ocular tolerability after a single drop of moxifloxacin ophthalmic solution 0.5%, in comparison with Tears Naturale Free (**F21**). However, Donnenfeld et al reported that normal young adult subjects receiving gatifloxacin ophthalmic solution

0.3% exhibited fewer increases in signs and symptoms of ocular intolerablity (i.e., conjunctival hyperemia, vascularity, ocular pain and irritation, and miosis) than those receiving moxifloxacin ophthalmic solution 0.5%.[4] The fluoroquinolone concentrations achieved in ocular tissues after topical instillation in animals and humans have been determined by a number of investigators and reviewed by Robertson et al.[16] The maximum concentrations achieved in the aqueous humor in rabbits ranged from 0.3 to 32.6 µg/mL depending on the dosage regimen and the fluoroquinolone. In humans, documented fluoroquinolone levels generally ranged from 0.11 to 2.28 µg/mL after topical treatment.[8] In those studies where various antibiotic products were compared, moxifloxacin generally achieved aqueous humor concentrations approximately 2 to 3 fold higher than gatifloxacin, ofloxacin or ciprofloxacin.[16] García-Sáenz et al reported aqueous humor concentrations of 2.33 µg/mL after 400 mg of moxifloxacin taken orally.[7] No reports of human cornea drug levels are available, but concentrations of moxifloxacin in rabbit cornea were 12.5 µg/g and 21.3 µg/g after a single dose or a 3-day t.i.d. regimen, respectively, as compared with 6.02 and 8.01 µg/g concentrations of ofloxacin by the same regimens and 4.85 µg/g for gatifloxacin t.i.d. (**F15**). Mather et al reported a mean rabbit cornea concentration of 158.66 µg/g at 60 minutes after topical dosing at 0, 5, 10, 60, 65, and 70 minutes (**F17**). In rabbit studies, drug concentrations in the cornea were found to be approximately 10–20 times those measured in aqueous humor, and if a similar relationship is present in humans, then cornea concentrations would be proportionately higher.

Corneal epithelial and endothelial cell morphologies were unchanged in volunteers receiving moxifloxacin ophthalmic solution 0.5% four times a day for 3 days after cataract surgery (**F22**). Moxifloxacin ophthalmic solution 0.5% and gatifloxacin ophthalmic solution 0.3% were tested on patients after laser-assisted *in situ* keratomileusis or laser epithelial keratomileusis surgeries had no effect on ophthalmic measures including quality of vision, comfort, and rates of corneal re-epithelialization.[5] Despite high tissue concentrations, no effects on postoperative corneal and conjunctival healing were reported. Corneal

F18 Rusinko A, May J, Liao J, et al: A study of the enhanced corneal penetration of moxifloxacin (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4907, 2004.

F19 Owen GR, Dembinska O, Stout KR, Mendiola MK: Corneal penetration and changes in corneal permeability of moxifloxacin versus gatifloxacin (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4910, 2004.

F20 Nguyen QH, Friedlaender MH, Sharf L, Breshears D: Objective and subjective measurement of drug toxicity (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4937, 2004.

F21 Wagner RS, D'Arienzo PA, Hallas SJ, et al: A comparative study in a normal pediatric population of the relative comfort of moxifloxacin 0.5% ophthalmic solution versus a tear substitute (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4913, 2004.

F22 Donaldson KE, Marangon FB, Schatz L, et al: Confocal analysis of the effects of moxifloxacin on the normal human cornea. Poster presented at Am Soc Cataract Refract Surg, May 1–5, 2004; San Diego CA.

epithelial healing time following photorefractive keratectomy was found to be better for subjects using moxifloxacin ophthalmic solution, 0.5% than for gatifloxacin ophthalmic solution, 0.3%, when given post-operatively, four times a day. Corneal epithelial defects had healed for 80% of eyes treated with moxifloxacin ophthalmic solution, 0.5%, as compared with 69% for gatifloxacin ophthalmic solution, 0.3% post-operative day 4. Epithelial defect size was also statistically significantly smaller for the moxifloxacin-treated eyes at day 4.[1] Thus, clinical experience shows that moxifloxacin ophthalmic solution 0.5% and other ophthalmic fluoroquinolones are safe and well tolerated in normal and postsurgical eyes.

Discussion

Fluoroquinolone antibiotics have proven safe and effective in both systemic and ophthalmic use. Nonclinical studies support the use of the ophthalmic products for the currently approved indications. Moxifloxacin is a fourth-generation fluoroquinolone with favorable characteristics such as potent efficacy and good ocular penetration. Systemic exposure (i.e., plasma drug levels) with topically applied fluoroquinolones is negligible, so untoward systemic effects are unlikely.

Nonclinical studies have established a high safety margin for ophthalmic moxifloxacin. Moxifloxacin ophthalmic solution given topically to rabbit eyes at concentrations of up to 3%, two drops three times a day for as long as 1 month, resulted in no significant ocular effects, including on corneal thickness, assessment by slit-lamp biomicroscopy or indirect ophthalmoscopy, and ocular histology. No systemic effects were evident as assessed by detailed in-life observations, clinical laboratory evaluations, and general histopathologic examinations. Extremely high topical doses of moxifloxacin ophthalmic solution instilled in normal monkey eyes for 3 months produced no ocular toxicity, with no abnormalities observed in corneal epithelium, stroma, or endothelium, and no significant systemic findings.

Some nonclinical *in vivo* studies have suggested potential effects of topical treatment with fluoroquinolones on healing rates in wounded cornea and differences between moxifloxacin and gatifloxacin, whereas other studies show similar wound healing, comparable with controls for both drugs. In general, it appears that there are no significant differences between the two drugs, and both drugs are safe for use under those conditions. *In vitro* studies of fluoroquinolones with human or rabbit corneal cells suggest that moxifloxacin has a low potential for dose- and time-dependent cytotoxicity.

Although some nonclinical studies have demonstrated inconsistent results with moxifloxacin ophthalmic solution 0.5% in the wounded cornea or wound healing, clinical reports consistently find no significant effects on postsurgical recovery with post-cataract, post–laser-assisted *in situ* keratomileusis, and post–laser epithelial keratomileusis use. Clinical studies have demonstrated high concentrations of moxifloxacin in aqueous humor after topical dosing. These high drug levels assure clinical efficacy, with concentrations of 10 to 30-fold higher moxifloxacin's MICs for common ocular pathogens.[18] Moxifloxacin ophthalmic solution 0.5% combines the therapeutic benefits of high antimicrobial efficacy and ocular tissue penetration with a favorable safety profile.

Method of Literature Search

A search of the MEDLINE and Toxline databases (1970–2005) was conducted using an online search tool (Endnote 5.0, ISI Researchsoft, Berkeley, CA). Search terms employed were *moxifloxacin, fluoroquinolone, ocular toxicity, ophthalmic toxicity,* and *eye toxicity*. All entries considered to be of significance were utilized, including those in the non-English literature if an English abstract was available. The reference section of each article was reviewed, and if it was felt to be of significance by adding additional data or refuting existing information, it was included. When necessary, data were computed from graphs and tables.

References

1. Burka JM, Bower KS, Vanrockel RC, Stutzman RD, Kuzmowych CP, Howard RS: The effect of fourth-generation fluoroquinolones gatifloxacin and moxifloxacin on epithelial healing following photorefractive keratectomy. Am J Ophthalmol 140:83–7, 2005
2. Clark L, Bezwada P, Hosoi K, et al: Comprehensive evaluation of ocular toxicity of topical levofloxacin in rabbit and primate models. J Toxicol Cutan Ocul Toxicol 23:1–18, 2004
3. Cutarelli PE, Lass JH, Lazarus HM, et al: Topical fluoroquinolones: antimicrobial activity and *in vitro* corneal epithelial toxicity. Curr Eye Res 10:557–63, 1991
4. Donnenfeld E, Perry HD, Chruscicki DA, et al: A comparison of the fourth-generation fluoroquinolones gatifloxacin 0.3% and moxifloxacin 0.5% in terms of ocular tolerability. Curr Med Res Opin 20:1753–8, 2004
5. Durrie DS, Trattler W: A comparison of therapeutic regimens containing moxifloxacin 0.5% ophthalmic solution and gatifloxacin 0.3% ophthalmic solution for surgical prophylaxis in patients undergoing LASIK or LASEK. J Ocular Pharm Ther 21:236–41, 2005
6. Fish DN: Fluoroquinolone adverse effects and drug interactions. Pharmacotherapy 21:S253–72, 2001
7. García-Sáenz MC, Arias-Puente A, Fresnadillo-Martinez MJ, et al: Human aqueous humor levels of oral ciprofloxacin, levofloxacin, and moxifloxacin. J Cataract Refract Surg 27:1969–1974, 2001
8. Hariprasad SM, Blinder KJ, Shah GK, et al: Penetration pharmacokinetics of topically administered 0.5% moxifloxacin ophthalmic solution in human aqueous and vitreous. Arch Ophthalmol 123:39–44, 2005

9. Herrygers LA, Noecker RJ, Lane LC, et al: Comparison of corneal surface effects of gatifloxacin and moxifloxacin using intensive and prolonged dosing protocols. Cornea 24:66–71, 2005

10. Ichijima H, Petroll WM, Jester JV, et al: Confocal microscopic studies of living rabbit cornea treated with benzalkonium chloride. Cornea 11:221–5, 1992

11. Kang J, Wang L, Chen X, et al: Interactions of a series of fluoroquinolone antibacterial drugs with human cardiac K+ channel HERG. Mol Pharmacol 59:122–6, 2001

12. Kovoor T, Kim A, McCulley J, et al: Evaluation of the corneal effects of topical ophthalmic fluoroquinolones using *in vivo* confocal microscopy. Eye Contact Lens 30:90–4, 2004

13. Matsumoto S, Stern M, Oda R, et al: Effect of ofloxacin on corneal epithelial wound healing evaluated by *in vitro* and *in vivo* methods. Drug Invest 6:96–103, 1993

14. Maurice D, Mishima S: Ocular pharmacokinetics. in Sears ML (ed): Pharmacology of the Eye. New York, Springer-Verlag, 1984, pp 19–116

15. Pollack G, McKelvie P, White J, et al: The *in vivo* effects of fluoroquinolones on rabbit corneas. Clin Experiment Ophthalmol 31:517–21, 2003

16. Robertson SM, Curtis MA, Schlech BA, Rusinko A, Owen GR, Dembinska O, Liao J, Dahlin DC: Ocular pharmaco, kinetics of moxifloxacin after topical treatment of animals and humans. Surv Ophthalmol 50(Suppl 1): S32–S45, 2005

17. Shell JW: Pharmacokinetics of topically applied ophthalmic drugs. Surv Ophthalmol 26:207–18, 1982

18. Stroman DW, Dajcs JJ, Cupp G, Schlech BA: *In vitro* and *in vivo* potency of moxifloxacin and moxifloxacin ophthalmic solution 0.5%, a new topical fluoroquinolone. Surv Ophthalmol 50(Suppl):S16–S31, 2005

19. von Keutz E, Schlüter G: Preclinical safety evaluation of moxifloxacin, a novel fluoroquinolone. J Antimicrob Chemother 43(Suppl B):91–100, 1999

Reprint address: David H. McGee, PhD, Alcon Research, Ltd., Mail Code R9-7, 6201 South Freeway, Fort Worth, TX 76134-2099, USA.

ELSEVIER

Clinical Safety of Moxifloxacin Ophthalmic Solution 0.5% (VIGAMOX®) in Pediatric and Nonpediatric Patients With Bacterial Conjunctivitis

Lewis H. Silver, PhD, Adrienne M. Woodside, PhD, and Dawnelle B. Montgomery, PharmD

Alcon Research, Ltd., Fort Worth, TX, USA

Abstract. Five independent, multicentered, double-masked, parallel, controlled studies were conducted to determine the safety of moxifloxacin ophthalmic solution 0.5% (VIGAMOX®) in pediatric and nonpediatric patients with bacterial conjunctivitis. Patients were randomized into one of two treatment groups in each study and received either moxifloxacin ophthalmic solution 0.5% b.i.d. or t.i.d. or a comparator. A total of 1,978 patients (918 pediatric and 1,060 nonpediatric) was evaluable for safety. The most frequent adverse event in the overall safety population was transient ocular discomfort, occurring at an incidence of 2.8%, which was similar to that observed with the vehicle. No treatment-related changes in ocular signs or visual acuity were observed with moxifloxacin ophthalmic solution 0.5%, except for one clinically relevant change in visual acuity. Thus, based upon a review of adverse events and an assessment of ocular parameters, moxifloxacin ophthalmic solution 0.5% formulated without the preservative, benzalkonium chloride, is safe and well tolerated in pediatric (3 days–17 years of age) and nonpediatric (18–93 years) patients with bacterial conjunctivitis. (Surv Ophthalmol 50:S55–S63, 2005. © 2005 Elsevier Inc. All rights reserved.)

Key words. adverse events · conjunctivitis · fluoroquinolone · moxifloxacin · ophthalmic · pediatric · safety · VIGAMOX®

Bacterial conjunctivitis is one of the most common ocular infections contracted by children; thus, the safety of a drug to treat ocular infections in children and in the overall population is of paramount importance.[4,5] Topical ocular fluoroquinolones, such as ciprofloxacin, have been used to safely treat conjunctivitis in pediatric and nonpediatric patients for many years.[7] Unfortunately, emerging bacterial resistance raises concerns about the effectiveness of current fluoroquinolones.[2,6,8] A solution of a new fourth-generation fluoroquinolone, moxifloxacin hydrochloride, is currently available and indicated for the treatment of bacterial conjunctivitis in patients 1 year and older.[9] Second- and third-generation fluoroquinolones target the enzymes deoxyribonucleic acid gyrase or topoisomerase IV to elicit their antibactericidal effect.[1] Unlike these earlier fluoroquinolones, the fourth-generation fluoroquinolones bind to both deoxyribonucleic acid gyrase and topoisomerase IV to provide a simultaneous attack on two different bacterial enzymes, thus requiring two independent mutations in the bacteria to develop resistance.[10]

Patient compliance with dosing of a topical antibiotic is necessary to effectively fight bacterial ocular

S55

infections. To enhance compliance, the currently marketed solution of moxifloxacin ophthalmic solution 0.5% (VIGAMOX®, Alcon Laboratories, Inc., Fort Worth, TX) is formulated at a physiological pH of 6.8 and does not include the preservative, benzalkonium chloride, which can have toxic effects.[11] Unlike other topical ophthalmic antibiotics, moxifloxacin has been formulated and packaged as a self-preserved formulation and requires no added preservative agent (e.g., benzalkonium chloride). Thus, establishing the safety of ophthalmic moxifloxacin in pediatric and nonpediatric patients enhances the clinical success of this fluoroquinolone in its self-preserved formulation. Church et al reviewed the clinical safety profile of moxifloxacin when administered systemically and found that it was safe and well tolerated in the treatment of respiratory tract infections.[3]

Five independent phase II and phase III multicenter, double-masked, parallel, well-controlled studies were conducted to evaluate the safety and efficacy of moxifloxacin ophthalmic solution 0.5%. The safety data were integrated across these five clinical trials and are presented in Tables 1–4. The discussion of the data focuses on a comparison of the integrated data for moxifloxacin ophthalmic solution 0.5% with

that of the vehicle because the number of patients receiving ofloxacin ophthalmic solution 0.3% (Ocuflox®, Allergan, Irvine, CA) and ciprofloxacin ophthalmic solution 0.3% (Ciloxan®, Alcon Laboratories) was significantly smaller and not evenly distributed between pediatric and nonpediatric patients.

Methods

STUDY DESIGN

Five clinical trials (phase II and phase III) were conducted to evaluate the efficacy and safety of a solution of moxifloxacin ophthalmic solution 0.5%. All studies were multicenter, double-masked, randomized, parallel, and controlled (active or vehicle). Patients of any race and either sex who were diagnosed with bacterial conjunctivitis were enrolled in the study; the minimum age requirement for each study varied, depending on the study design (Table 5). The primary efficacy endpoint was the clinical cure rate for bulbar conjunctival injection and conjunctival discharge. An institutional review board approved each study before its initiation.

Patients with any of the following conditions were excluded from study participation: contact lens wear

TABLE 1

Most Frequent Adverse Events in Pediatric versus Nonpediatric Patients

| | Moxifloxacin 0.5% b.i.d. or t.i.d. | | | | | | Vehicle b.i.d. or t.i.d. | | | | | |
| | Pediatrics (≤17 Years) (N = 462) | | Nonpediatrics (≥18 Years) (N = 531) | | Total (N = 993) | | Pediatrics (≤17 Years) (N = 323) | | Nonpediatrics (≥18 Years) (N = 283) | | Total (N = 606) | |
Adverse Event	N	%	N	%	N	%	N	%	N	%	N	%
Ocular												
Ocular discomfort	9	1.9	19	3.6	28	2.8	7	2.2	6	2.1	13	2.1
Keratitis	3	0.6	5	0.9	8	0.8	2	0.6	6	2.1	8	1.3
Conjunctivitis	5	1.1	5	0.9	10	1.0	6	1.9	2	0.7	8	1.3
Ocular pruritus	3	0.6	9	1.7	12	1.2	1	0.3	4	1.4	5	0.8
Visual acuity decrease	2	0.4	6	1.1	8	0.8	3	0.9	5	1.8	8	1.3
Subconjunctival hemorrhage	4	0.9	2	0.4	6	0.6	2	0.6	2	0.7	4	0.7
Blepharitis	2	0.4	4	0.8	6	0.6	1	0.3	2	0.7	3	0.5
Nonocular												
Infection	12	2.6	8	1.5	20	2.0	15	4.6	1	0.4	16	2.6
Headache	3	0.6	9	1.7	12	1.2	3	0.9	16	5.7	19	3.1
Fever	10	2.2	0	0.0	10	1.0	5	1.5	1	0.4	6	1.0
Cold syndrome	3	0.6	3	0.6	6	0.6	2	0.6	2	0.7	4	0.7
Vomiting	6	1.3	0	0.0	6	0.6	5	1.5	4	1.4	9	1.5
Increased cough†	15	3.2	3	0.6	18	1.8	9	2.8	5	1.8	14	2.3
Rhinitis	11	2.4	3	0.6	14	1.4	11	3.4	4	1.4	15	2.5
Pharyngitis	4	0.9	4	0.8	8	0.8	7	2.2	6	2.1	13	2.1
Otitis media	14	3.0	1	0.2	15	1.5	10	3.1	1	0.4	11	1.8

* Occurring at an incidence of >5 reports (0.5%) in the moxifloxacin total column. Table includes related and non-related adverse events.

† Worsening of existing cough or development of a cough. The COSTART dictionary does not have a separate code for the development of a cough.

TABLE 2

Most Frequent[*] Related Adverse Events

| | Moxifloxacin 0.5% b.i.d. or t.i.d. | | | | | | Vehicle b.i.d. or t.i.d. | | | | | |
| | Pediatrics (≤17 Years) (N = 462) | | Nonpediatrics (≥18 Years) (N = 531) | | Total (N = 993) | | Pediatrics (≤17 Years) (N = 323) | | Nonpediatrics (≥18 Years) (N = 283) | | Total (N = 606) | |
Adverse Event	N	%	N	%	N	%	N	%	N	%	N	%
Ocular												
Ocular discomfort	9	1.9	18	3.4	27	2.7	5	1.5	5	1.8	10	1.7
Ocular pruritus	2	0.4	4	0.8	6	0.6	1	0.3	1	0.4	2	0.3
Ocular hyperemia	3	0.6	0	0.0	3	0.3	0	0.0	0	0.0	0	0.0
Ocular pain	0	0.0	3	0.6	3	0.3	0	0.0	0	0.0	0	0.0
Nonocular												
Headache	1	0.2	1	0.2	2	0.2	0	0.0	2	0.7	2	0.3
Taste perversion	1	0.2	3	0.6	4	0.4	0	0.0	0	0.0	0	0.0

[*] Occurring at an incidence of >1 report (0.1%) in the moxifloxacin total column. A generous cutoff was chosen due to the low frequency of related adverse events.

during the study; vision not correctable to 0.6 logarithm of the minimum angle of resolution (logMAR) or better (children younger than 3 years must be able to fix and follow); abnormal findings on fundus examination or presence of active inflammation in the cornea, iris, or anterior chamber at the day 1 visit; an allergy or hypersensitivity to fluoroquinolones or benzalkonium chloride; any current immunosuppressive disorder; or a suspected fungal, viral, or *Acanthamoeba* infection. In addition, patients who had any systemic or ocular disorder, complicating factors, or structural abnormality that would affect the study outcome were excluded from participation. Patients taking any of the following medications were also excluded from participation: any preserved topical ocular medications; a topical ocular antibacterial within 24 hours of the study; an oral antibacterial within 72 hours of the study; a systemic steroid within 14 days of the study; a systemic nonsteroidal antiinflammatory within 24 hours of the study (unless it was a steady regimen continuing for at least 2

months); or any other investigational study medication or immunosuppressive therapy (including chemotherapy). Also excluded from the study were those women who were pregnant or nursing. Females of childbearing potential could participate if they had a negative urine pregnancy test before randomization and used adequate birth control throughout the study.

Patients satisfying the exclusion and inclusion criteria rated their ocular symptoms on a four-point scale, and investigators assessed the ocular signs of the patient. Each patient underwent an ophthalmic examination that may have included a measurement of visual acuity and an assessment of ocular signs. Not all studies measured visual acuity or ocular signs (Table 5). Up to three microbiological specimens were collected from the affected eye(s) of the patient. Finally, a dilated or undilated fundus examination was conducted for each patient, except in study C-01-34. All patients (or their legal guardians) gave written informed consent before study participation.

TABLE 3

Additional Safety Assessments: Visual Acuity and Changes in Ocular Signs

| | Clinically Relevant Changes from Baseline to Any Visit | | | | |
| | LogMAR or Snellen Visual Acuity | | Changes in Ocular Signs | | |
Treatment	Total N	≥3 Line Decrease [N (%)]	Total N	Cornea [N (%)]	Iris/Anterior Chamber [N (%)]
Moxifloxacin 0.5%	737	7 (0.9)	807	4 (0.5)	0
Ofloxacin 0.3%	269	0	277	8[*] (2.9)	1 (0.4)
Vehicle	448	5 (1.1)	535	5 (0.9)	2 (0.4)

logMAR = logarithm of the minimum angle of resolution.
Visual acuity assessed in C-00-02, C-00-46, C-00-55, and C-01-66. Ocular signs evaluated in C-00-46, C-00-55, and C-01-66. Thus, no data for visual acuity and ocular signs are available for ciprofloxacin 0.3%. Patients 3 years and younger were excluded from visual acuity assessments.
[*] One person receiving ofloxacin 0.3% experienced a treatment-related change in the cornea.

TABLE 4

Most Frequent[*] Adverse Events by Age

	Moxifloxacin 0.5% (t.i.d. or b.i.d.)					
Adverse Event	Newborns < 28 Days (N = 100) [N (%)]	Infants and Toddlers 28 Days 23 Months (N = 66) [N (%)]	Children 2–11 Years (N = 237) [N (%)]	Adolescents 12–17 Years (N = 59) [N (%)]	Adults 18–64 Years (N = 477) [N (%)]	Elderly ≥ 65 Years (N = 54) [N (%)]
Ocular						0
Blepharitis	0	0	1 (0.4)	1 (1.7)	4 (0.8)	0
Conjunctivitis	0	1 (1.5)	3 (1.3)	1 (1.7)	5 (1.0)	0
Dry eye	0	0	0	0	4 (0.8)	0
Keratitis	0	0	1 (0.4)	2 (3.4)	5 (1.0)	0
Ocular discomfort	0	0	7 (3.0)	2 (3.4)	19 (4.0)	0
Ocular pain	0	0	0	0	5 (1.0)	0
Ocular pruritus	0	0	2 (0.8)	1 (1.7)	9 (1.9)	0
Visual acuity decrease	0	0	2 (0.8)	0	5 (1.0)	1 (1.9)
Nonocular						
Fever	1 (1.0)	4 (6.1)	5 (2.1)	0	0	0
Headache	0	0	0	3 (5.1)	9 (1.9)	0
Increased cough[†]	1 (1.0)	3 (4.5)	11 (4.6)	0	3 (0.6)	0
Infection	0	2 (3.0)	10 (4.2)	0	8 (1.7)	0
Otitis media	0	5 (7.6)	9 (3.8)	0	1 (0.2)	0
Pharyngitis	0	0	2 (0.8)	2 (3.4)	4 (0.8)	0
Rhinitis	2 (2.0)	3 (4.5)	6 (2.5)	0	3 (0.6)	0

Adverse Events listed alphabetically.
[*] Four or more reports occurring in at least one age group. A more generous cutoff was chosen due to the smaller Ns in the age groups relative to Table 1. Table includes related and non-related adverse events.
[†] Worsening of existing cough or development of a cough. The COSTART dictionary does not have a separate code for the development of a cough.

After the screening examination, qualified patients were randomized to receive either moxifloxacin ophthalmic solution 0.5% or a comparator (i.e., vehicle, ofloxacin ophthalmic solution 0.3%, or ciprofloxacin ophthalmic solution 0.3%). Study C-00-46 compared moxifloxacin ophthalmic solution 0.5% with ofloxacin ophthalmic solution 0.3%, and study C-01-34 compared moxifloxacin ophthalmic solution 0.5% with ciprofloxacin ophthalmic solution 0.3%, whereas the other three studies were vehicle controlled (Table 5). Study medication was first administered by a member of the investigator's staff at the conclusion of the eligibility visit (day 1). In the phase III studies (C-00-46, C-00-55, C-01-34, and C-01-66), after the day 1 visit patients were instructed to administer one drop of the masked medication

TABLE 5

Designs of Clinical Studies Including Measured Safety Parameters

	C-00-02	C-00-46	C-00-55	C-01-34	C-01-66
Age range	1–89 years	1–85 years	1 month–89 years	2–30 days	48 days–93 years
Duration of treatment	3 days	4 days	4 days	4 days	4 days
Duration of assessment[*]	7 days	9 days	9 days	9 days	9 days
Moxifloxacin group	0.5% b.i.d.	0.5% t.i.d.	0.5% t.i.d.	0.5% t.i.d.	0.5% t.i.d.
Comparator group	Vehicle b.i.d.	Ofloxacin 0.3% q.i.d.	Vehicle t.i.d.	Ciprofloxacin 0.3% t.i.d.	Vehicle t.i.d.
No. of study sites	20	15	32	32	31
Location of study	USA	India	USA	USA	USA
Adverse events	Yes	Yes	Yes	No	Yes
Visual acuity	Yes	Yes	Yes	No	Yes
Ocular signs	No[†]	Yes	Yes	Yes	Yes

[*] Total days of enrollment in the study.
[†] In this study, assessment of ocular signs was an efficacy parameter only. In subsequent studies, the measurement of ocular signs was expanded to include safety assessments of the cornea and iris/anterior chamber.

in the conjunctival sac of both eyes t.i.d. for 4 days' total exposure. Patients were observed for a total of 9 days. In the phase II study, C-00-02, patients received either one or two drops of medication b.i.d. for 3 days' total exposure, and the patients were observed for a total of 7 days. A summary of the demographics of the patients integrated across the five studies is shown in Table 6.

SAFETY ASSESSMENTS

Safety data were pooled across the five clinical studies. The evaluation of safety was conducted on all patients who were randomized into the study and received at least one dose of the study drug. Adverse events were defined as any change (expected or unexpected) in a patient's ophthalmic and/or systemic health that occurred after initiation of study treatment. These changes included any changes in visual acuity or ocular signs as defined in each study protocol. The principal investigator and a medical monitor independently assessed causality of these events, and the events were coded using a modified COSTART dictionary.

The principal investigator assessed best corrected visual acuity at the day 1 (baseline) visit and at all subsequent visits for patients older than 3 years. In study C-01-34, visual acuity was not measured, as the study involved patients younger than 1 month.

For all other patients, the logMAR (or Snellen) procedure was used to assess visual acuity. The maximum change in visual acuity for the worse eye in each patient (i.e., the eye with greatest decrease in visual acuity) was calculated as the change in logMAR lines (0.1 units = 1 logMAR line) from baseline to any visit. For pediatric patients whose visual acuity could not be determined using logMAR (or Snellen), an age-appropriate measurement method, either HOTV or Crowded Symbols, was used to calculate the maximum change in visual acuity. Clinically relevant changes in visual acuity were defined as a decrease of 3 or more logMAR (or Snellen) lines from baseline and resulted in the reporting of an adverse event.

Ocular signs were assessed in studies C-00-46, C-00-55, and C-01-66. Ocular signs (cornea and iris/anterior chamber) were assessed by the principal investigator using a penlight, ophthalmoscope, or slit lamp at day 1 (baseline) and at all subsequent (scheduled or unscheduled) visits. Clinically relevant changes in ocular signs were defined as any increase in parameter score from baseline (Table 7) and resulted in the reporting of an adverse event.

Results

OVERALL SAFETY POPULATION

The safety profile of moxifloxacin ophthalmic solution 0.5% was evaluated in 1,978 patients across five

TABLE 6
Demographics of Safety Population

Demographic Parameter	Moxifloxacin 0.5% (N = 993)		Ofloxacin 0.3% (N = 277)		Ciprofloxacin 0.3% (N = 102)		Vehicle (N = 606)	
	N	%	N	%	N	%	N	%
Sex								
Male	495	49.8	188	67.9	57	55.9	256	42.2
Female	498	50.2	89	32.1	45	44.1	350	57.8
Race								
White	500	50.4	0	0	70	68.6	409	67.5
Black	51	5.1	0	0	3	2.9	53	8.7
Other*	442	44.5	277	100.0	29	28.4	144	23.8
Iris color†								
Brown	321	32.3	276	99.6	34	33.3	300	49.5
Blue	224	22.6	0	0	48	47.1	171	28.2
Other	446	44.9	1	0.4	20	19.6	135	22.3
Age								
Newborn infants (<28 days)	100	10.1	0	0	97	95.1	0	0
Infants and toddlers (28 days–23 months)	66	6.6	4	1.4	5	4.9	63	10.4
Children (2–11 years)	237	23.9	21	7.6	0	0	222	36.6
Adolescents (12–17 years)	59	5.9	6	2.2	0	0	38	6.3
Adults (18–64 years)	477	48.0	220	79.4	0	0	260	42.9
Elderly (≥65 years)	54	5.4	26	9.4	0	0	23	3.8

* Moxifloxacin 0.5% other includes 290 Asian, 129 Hispanic, and 23 other. Ofloxacin 0.3% other includes 277 Asian. Ciprofloxacin 0.3% other includes 26 Hispanic and 3 other. Vehicle other includes 13 Asian, 118 Hispanic, and 13 other.
† Two patients in the moxifloxacin 0.5% treatment group have missing iris color data.

TABLE 7

Scoring of Ocular Signs Parameters

	Specifications	Definition of Terms	
		Normal (0)	Abnormal (1)
Cornea	Includes all corneal layers	Absence of active inflammation or active structural change	Presence of active inflammation or active structural change including focal scarring and fine deposition
Iris/anterior chamber	Includes evaluation of anterior chamber and its surrounding structure	Absence of active inflammation	Presence of active inflammation

clinical studies who received at least one dose of study medication. Moxifloxacin ophthalmic solution 0.5% was safe and well tolerated in the overall patient population. No serious adverse events assessed as related to therapy were reported. Overall, adverse events assessed as related to moxifloxacin ophthalmic solution 0.5% occurred in 4.7% of the patients, whereas 2.6% of the patients experienced adverse events assessed as related to the vehicle (data not shown). Adverse events in the moxifloxacin ophthalmic solution 0.5% and vehicle groups that were related to study therapy were generally mild in intensity and usually resolved on their own or with treatment. In addition, adverse events in the active-controlled studies (Table 8) were similar when comparing moxifloxacin ophthalmic solution 0.5% with ofloxacin ophthalmic solution 0.3% or with ciprofloxacin ophthalmic solution 0.3%.

As presented in Table 1, the most frequent adverse event (related and not related combined) in the overall safety population receiving moxifloxacin ophthalmic solution 0.5% was ocular discomfort (i.e., transient burning and stinging), which occurred at an incidence of 2.8% and was similar to that observed with the vehicle group (2.1%). In addition, transient ocular discomfort was the most common treatment-related event (Table 2) in both the moxifloxacin ophthalmic solution 0.5% group (2.7%) and the vehicle group (1.7%). These events of ocular discomfort were mostly mild in intensity, usually resolved without treatment, and rarely prevented a patient from completing the study.

The most frequent nonocular adverse event (related and not related combined) in patients receiving moxifloxacin ophthalmic solution 0.5% was general infection (Table 1), which occurred in 2.0% of the

TABLE 8

Most Frequent[*] Adverse Events in the Active-Controlled Studies (C-00-46 and C-01-34)

	C-00-46[†]				C-01-34[‡]			
	Moxifloxacin 0.5% t.i.d. (N = 277)		Ofloxacin 0.3% q.i.d. (N = 277)		Moxifloxacin 0.5% t.i.d. (N = 107)		Ciprofloxacin 0.3% t.i.d. (N = 102)	
Adverse Event	N	%	N	%	N	%	N	%
Ocular								
Ocular discomfort	4	1.4	1	0.4	0	0.0	0	0.0
Keratitis	3	1.1	3	1.1	0	0.0	0	0.0
Corneal infiltrate	2	0.7	3	1.1	0	0.0	0	0.0
Tearing	0	0.0	0	0.0	3	2.8	2	2.0
Ocular hyperemia	1	0.4	0	0.0	2	1.9	1	1.0
Nonocular								
Rash	0	0.0	0	0.0	3	2.8	3	2.9
Rhinitis	0	0.0	1	0.4	2	1.9	4	3.9
Surgical/medical procedure[§]	0	0.0	0	0.0	2	1.9	3	2.9
Eructation	0	0.0	0	0.0	2	1.9	0	0.0

[*] Occurring at an incidence of >1 report in either moxifloxacin column. A generous cutoff was chosen due to the low frequency of adverse events in the active-controlled studies. Table includes related and non-related adverse events.

[†] Most patients were nonpediatric patients.

[‡] All patients were pediatric patients (2–30 days old).

[§] For moxifloxacin 0.5%, 1 case of circumcision and 1 case of frenulectomy. For ciprofloxacin 0.3%, 3 cases of circumcision.

patients. This incidence of infection was similar to that seen with the vehicle (2.6%). These reported adverse events of infection were not related to the study drug (Table 2). Other frequently reported non-ocular adverse events (i.e., increased cough, rhinitis, and otitis media) were not related to moxifloxacin and occurred at a lower incidence than in the vehicle.

After an assessment of visual acuity, seven patients (an incidence of 0.9%) receiving moxifloxacin ophthalmic solution 0.5% exhibited a clinically relevant decrease in visual acuity (Table 3), which is similar to that for the vehicle (1.1%). Clinically relevant changes in visual acuity were defined as a decrease of 3 or more logMAR (or Snellen) lines from baseline (day 1 visit). No patient discontinued from the study due to a change in visual acuity. For patients exposed to moxifloxacin ophthalmic solution 0.5%, no changes in visual acuity were related to the study drug, with one exception. In this incident, a 4-year-old child was uncooperative in follow-up visual acuity measurements, and the event was conservatively reported as related. No clinically relevant-treatment-related changes in ocular signs (cornea or iris/anterior chamber) were observed with moxifloxacin ophthalmic solution 0.5%.

PEDIATRIC POPULATION

In the five clinical studies, the safety profile of a solution of moxifloxacin ophthalmic solution 0.5% was evaluated in 462 pediatric patients (3 days–17 years old) who received at least one dose of study medication. Moxifloxacin ophthalmic solution 0.5% was safe and well tolerated in pediatric patients, including all age categories: newborns (0–27 days), infants and toddlers (28 days–23 months), children (2–11 years), and adolescents (12–17 years). No serious adverse events assessed as related to therapy were reported in any pediatric patient. A small incidence of pediatric patients (3.5%) who received moxifloxacin ophthalmic solution 0.5% experienced a treatment-related adverse event, with a similar incidence of 2.8% observed for those receiving the vehicle (data not shown). Treatment-related adverse events experienced by pediatric patients were generally mild in intensity and usually resolved on their own.

As seen in the overall safety population, the most common ocular adverse event (related and not related combined) experienced by pediatric patients in the moxifloxacin ophthalmic solution 0.5% group was ocular discomfort (Table 1), which occurred in 1.9% of the patients, with a similar incidence of 2.2% reported for those in the vehicle group. All adverse events of ocular discomfort experienced by pediatric patients receiving moxifloxacin ophthalmic solution 0.5% were related to the study drug, whereas five of

seven adverse events of discomfort for those in the vehicle group were treatment-related adverse events. Although ocular discomfort was the most common treatment-related adverse event, the events were generally mild, usually resolved on their own within minutes of onset, and did not interrupt patient participation in the study. Most ocular adverse events (related and not related combined) were not reported in more than three pediatric patients, except ocular discomfort, conjunctivitis, subconjunctival hemorrhage, and tearing.

The most common systemic adverse event (related and not related combined) experienced by pediatric patients in the moxifloxacin ophthalmic solution 0.5% and vehicle groups was increased cough (includes the development of a cough; see Table 1 footnote), which occurred in 3.2% and 2.8% of patients, respectively (Table 1). For patients exposed to moxifloxacin ophthalmic solution 0.5%, most of the frequently reported systemic adverse events (including increased cough, infection, rhinitis, and otitis media) were assessed as not related to the study drug.

NONPEDIATRIC POPULATION

In the five clinical studies, the safety profile of moxifloxacin ophthalmic solution 0.5% was evaluated in 531 nonpediatric (i.e., adult and elderly) patients who were 18 to 93 years old and received at least one dose of study medication. Moxifloxacin ophthalmic solution 0.5% was safe and well tolerated in adult (18–64 years old) and elderly (65 years and older) patients. No serious adverse event assessed as related to therapy was reported in any adult or elderly patient. Adult and elderly patients who received moxifloxacin ophthalmic solution 0.5% experienced treatment-related adverse events (5.8%) at an incidence similar to that of those who received vehicle (3.9%; data not shown).

As seen in the overall safety population, the most common adverse event experienced by adult and elderly patients exposed to moxifloxacin ophthalmic solution 0.5% was ocular discomfort, which occurred in 3.6% of the patients (Table 1) versus an incidence of 2.1% for patients receiving the vehicle. These events of ocular discomfort were mild in intensity (except for one incident that was moderate), resolved without treatment, and did not prevent any patient from completing the study with one exception. All events of ocular discomfort in the nonpediatric population were related to the study drug, with one exception in both the moxifloxacin ophthalmic solution 0.5% group and the vehicle group. Nonpediatric patients who complained of ocular discomfort in the moxifloxacin ophthalmic solution 0.5% group (Table 4) were only in the adult age group and not

the elderly group. Also, although visual acuity decrease was reported in 1.1% of the nonpediatric patients receiving moxifloxacin ophthalmic solution 0.5% (and at an incidence of 1.8% in the vehicle group), none of these were treatment-related. In fact, besides ocular discomfort, ocular pruritus, and ocular pain (Table 2), no treatment-related ocular adverse event was reported in more than one nonpediatric patient across all five studies.

Common systemic adverse events (related and not related combined) experienced by nonpediatric patients (Table 1) who received moxifloxacin ophthalmic solution 0.5% were general infection (1.5%) and headache (1.7%), which all occurred in adult patients (Table 4). With one exception of headache, these systemic events were not related to the study drug for patients exposed to moxifloxacin ophthalmic solution 0.5%. Nonpediatric patients exposed to the vehicle reported an incidence of 0.4% for infection and 5.7% for headache, which were mostly not related to the study drug.

Conclusion

Because the use of topical fourth-generation fluoroquinolones is relatively new, it is important to establish the safety and tolerability of moxifloxacin ophthalmic solution 0.5% formulated without benzalkonium chloride. Thus, the safety of moxifloxacin ophthalmic solution 0.5% was evaluated against a comparator (i.e., ofloxacin ophthalmic solution 0.3%, ciprofloxacin ophthalmic solution 0.3%, or vehicle) in clinical studies that included 1,978 patients pooled across five studies. No serious treatment-related adverse events were reported in these studies. In fact, no adverse event (treatment-related or not related) was reported across the five studies at an incidence greater than 3.0% in the moxifloxacin ophthalmic solution 0.5% group. Ocular adverse events in the overall population, particularly those related to the study drug, were generally mild in intensity and usually resolved without intervention. A review of the adverse event profile, visual acuity assessments, and ocular signs parameters for patients enrolled in the five clinical studies demonstrated that the safety and tolerability of a solution of moxifloxacin ophthalmic solution 0.5% was similar to that of the vehicle. In addition, no safety concerns were identified when comparing moxifloxacin ophthalmic solution 0.5% with ofloxacin ophthalmic solution 0.3% or ciprofloxacin ophthalmic solution 0.3%.

The most common treatment-related adverse event in the overall safety population, including both pediatric and nonpediatric patients, was transient ocular discomfort. Although a solution of moxifloxacin ophthalmic solution 0.5% is the highest concentration

of a fourth-generation fluoroquinolone currently available, the incidence of transient discomfort in those receiving moxifloxacin ophthalmic solution 0.5% t.i.d. was similar to that seen in the vehicle group. These events of ocular discomfort were usually mild in intensity and generally resolved on their own after a few minutes. From product launch in May 2003 through December 31, 2004, only 12 postmarketing adverse event reports of eye irritation have been received out of more than 3.6 million units of moxifloxacin ophthalmic solution 0.5% (VIGAMOX®) sold, confirming the safety and tolerability of this product already demonstrated in the clinical studies. Besides transient ocular discomfort, ocular pruritus and taste perversion were the only treatment-related adverse events reported in more than three patients receiving moxifloxacin ophthalmic solution 0.5% in the overall safety population across the five clinical studies.

Because bacterial conjunctivitis is an infection commonly diagnosed in pediatric patients, the safety of moxifloxacin ophthalmic solution 0.5% was evaluated in individuals as young as 3 days.[4] The safety profile of moxifloxacin ophthalmic solution 0.5% in pediatric patients was similar to that observed in nonpediatric patients and in the overall safety population. In particular, ocular adverse events were detected in the pediatric patients, including newborns, at an incidence less than 2.5%, which demonstrates the safety and tolerability of moxifloxacin ophthalmic solution 0.5% even in the youngest pediatric patients. Some nonocular adverse events were reported at slightly higher incidences relative to ocular events; however, these events were generally not related to the study drug. In fact, besides ocular discomfort and ocular hyperemia (data not shown), no treatment-related adverse event occurred in more than two pediatric patients exposed to moxifloxacin ophthalmic solution 0.5% across the five clinical studies.

In summary, data integrated across five well-controlled clinical studies demonstrate that moxifloxacin ophthalmic solution 0.5% presents no safety concerns for patients, including newborns and infants/toddlers. In addition, the safety of moxifloxacin ophthalmic solution 0.5% did not significantly differ from that of ofloxacin ophthalmic solution 0.3%, ciprofloxacin ophthalmic solution 0.3%, or the vehicle. Thus, moxifloxacin ophthalmic solution 0.5% dosed t.i.d. is safe and well tolerated in pediatric and nonpediatric patients.

Methods of Literature Search

References cited in this article were identified from searches of the following computer-based databases:

MEDLINE, reference lists of review articles, and Association for Research in Vision and Ophthalmology abstracts (2001–2005). Key words employed were VIGAMOX®, *moxifloxacin*, *fluoroquinolone*, *pediatric*, *adverse events*, and *safety*. No non-English articles are cited.

References

1. Blondeau JM: Fluoroquinolones: mechanism of action, classification, and development of resistance. Surv Ophthalmol 49(Suppl 2):S73–8, 2004
2. Chaudhry NA, Flynn HW, Murray TG, et al: Emerging ciprofloxacin-resistant *Pseudomonas aeruginosa*. Am J Ophthalmol 128:509–10, 1999
3. Church D, Haverstock D, Andriole VT: Moxifloxacin: a review of its safety profile based on worldwide clinical trials. Todays Therapeut Trends 18(3):205–33, 2000
4. Fisher MC: Conjunctivitis in children. Pediatr Clin North Am 34:1447–56, 1987
5. Gigliotti F, Hendley JO, Morgan J, et al: Efficacy of topical antibiotic therapy in acute conjunctivitis in children. J Pediatr 104:623–6, 1984
6. Goldstein MH, Kowalski RP, Gordon YJ: Emerging fluoroquinolone resistance in bacterial keratitis: a 5-year review. Ophthalmology 106:1313–18, 1999
7. Gross RD, Hoffman RO, Lindsay RN: A comparison of ciprofloxacin and tobramycin in bacterial conjunctivitis in children. Clin Pediatr (Phila) 36:445–8, 1997
8. Kowalski RP, Pandya AN, Karenchak LM, et al: An *in vitro* resistance study of levofloxacin, ciprofloxacin, and ofloxacin using keratitis isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Ophthalmology 108:1826–9, 2001
9. Mather R, Karenchak LM, Romanowski EG, Kowalski RP: Fourth generation fluoroquinolones: new weapons in the arsenal of ophthalmic antibiotics. Am J Ophthalmol 133: 463–6, 2002
10. Schedletzky H, Wiedemann B, Heisig P: The effect of moxifloxacin on its target topoisomerases from *Escherichia coli* and *Staphylococcus aureus*. J Antimicrob Chemother 43:31–7, 1999
11. Tripathi BJ, Tripathi RC, Kolli SP: Cytotoxicity of ophthalmic preservatives on human corneal epithelium. Lens Eye Toxic Res 9:361–75, 1992

ELSEVIER

CONCLUSION

Future of Ophthalmic Anti-infective Therapy and the Role of Moxifloxacin Ophthalmic Solution 0.5% (VIGAMOX®)

Barry A. Schlech, PhD,[1] and Joseph Blondeau, PhD[2]

[1]Alcon Research, Ltd., Fort Worth, Texas, USA; and [2]Department of Microbiology, Royal University Hospital, Saskatoon, Saskatchewan, Canada

Abstract. The vintage antibiotics that were available in the 1950s–1980s were sometimes toxic, had limited spectra, and were bacteriostatic agents, and they have been replaced by significantly broader-spectrum therapies. We ask more of our future antibiotic products for ophthalmology: they must be 1) broad spectrum, 2) convenient to use, 3) useful prophylactically, 4) effective therapeutically, 5) benzalkonium chloride–free, 6) comfortable, and 7) nontoxic. The emergence of antibiotic resistance has focused us on more potent agents effective against resistant strains of bacteria. Fluoroquinolones have become a dominant family of ophthalmic antibiotics. But even the older fluoroquinolones (e.g., ofloxacin, ciprofloxacin) have lost much of their effectiveness against some important ocular isolates. Considering all of the characteristics for an ideal ophthalmic antibiotic product available today, moxifloxacin ophthalmic solution 0.5% represents a primary antibiotic product of choice for treating and preventing ophthalmic infections. (Surv Ophthalmol 50:S64–S67, 2005. © 2005 Elsevier Inc. All rights reserved.)

Key words. benzalkonium chloride • ciprofloxacin • fluoroquinolones • gatifloxacin • levofloxacin • moxifloxacin • ofloxacin • ophthalmic therapy • VIGAMOX®

Product Evolution and Demands

Gone are the days in the 1950s and 1960s when there was a wide range of choices for antibiotic therapy. Eye care practitioners no longer use sulfonamides, chloramphenicol, polymyxin, or bacitracin to treat ophthalmic infections. The use of aminoglycosides (neomycin, gentamicin, tobramycin) is waning as better agents in the fluoroquinolone family become available. These 1950s vintage antibiotics were sometimes toxic, had limited spectra, were bacteriostatic agents, and have been replaced by significantly broader-spectrum therapy. We ask more of our

future antibiotic products for ophthalmology. They must be very effective against a wide range of infectious agents. They must be convenient to use for the surgeon and patient. They must cure quickly or sterilize the surgical eye area effectively. They should be able to be used prophylactically to prevent infections and therapeutically to cure infections. They must be able to be used in a wide variety of people, from neonates to geriatric patients. They must be broad enough to be used prophylactically and strong enough to cure serious, specific infections. Their dosage regimens must be simple, yet effective. They

S64

must be comfortable and nontoxic to the eye. They should be useful in treating or preventing a wide range of ocular infections (e.g., conjunctivitis, keratitis, endophthalmitis, blepharitis, dacryocystitis).

Growing Antibiotic Resistance

Growing microbial resistance to current antibacterial agents and widening gaps in antibiotic coverage create a need for a more potent and genetically smart fluoroquinolone. When ciprofloxacin, the first ocular fluoroquinolone, became available for ophthalmic use roughly a decade and a half ago, there was tremendous excitement. This was our knockout punch in the fight to prevent ocular infection, especially after cataract and refractive surgery. Today, however, our most impressive weapon has lost some of its punch.[13,16,24] Several microorganism groups have developed resistance to ciprofloxacin and its sister fluoroquinolones, ofloxacin and levofloxacin, more quickly than imagined, and resistance levels are increasing each year (F1).[16]

The bacteria hold most of the cards for the future. They will evolve and respond to their environment and produce progeny that will be resistant to today's antibiotics. Humans can only try to keep abreast of these clever creatures. Abandoning the old antibiotics and taking on the new is the only way to keep abreast of and even stop resistant trends. Continuing to use older, previous-generation antibiotics will only facilitate the continued development of resistant strains.[11] To our knowledge, there are no studies that prove or suggest topical application of moxifloxacin has the potential to induce microbial resistance distal to the site of instillation.

Conjunctivitis

Conjunctivitis occurs worldwide and affects people of all ages, all social strata, and both sexes. It has been cited as one of the most frequent causes of self-referral in the practice of comprehensive ophthalmology.[8,12,14,18] According to the American Academy of Ophthalmology (F2), conjunctivitis infrequently causes permanent visual loss or structural damage, but the economic impact of the disease in terms of lost work time, although undocumented, is doubtless considerable.

Fluoroquinolones

The fluoroquinolones are an evolving and powerful class of broad-spectrum antimicrobial agents used in the prevention and treatment of a variety of ocular infections; however, resistance to currently available agents in the class has been emerging among ocular pathogens.[2,4] They interfere with bacterial deoxyribonucleic acid synthesis, and newer generations of these compounds have improved broad-spectrum coverage. The topical fourth-generation fluoroquinolones, moxifloxacin and gatifloxacin, were approved in 2003 by the US Food and Drug Administration for ocular indications. These antibiotics represent the most advanced group of compounds within the class, offer a unique dual-binding mechanism of action in gram-positive organisms, and have activity against otherwise resistant species.[4] Recent reports (F3) have indicated that the fourth-generation fluoroquinolones, moxifloxacin and gatifloxacin, are more effective than earlier generations of fluoroquinolones and tobramycin, based on minimum inhibitory concentrations (MICs) and susceptibility results. Several recent *in vivo* studies using prophylactic models with rabbits have shown the potency of these antibiotics in preventing infections by common pathogens.[3,9,17] Although further clinical evidence of their efficacy in prophylaxis and treatment of human ocular infections is required, there is a growing need for compounds with this potential to combat emerging resistance.[4,6]

Benzalkonium Chloride–Free Products

Agents that are innately antibacterial, such as antibiotics, like the fluoroquinolones, have the opportunity of being formulated in multiple-dose containers without added antimicrobial preservative agents, such as benzalkonium chloride. This preservative has served the ophthalmic community well over the last 50 years and is still required for preserving antiglaucoma and other ophthalmic products. But researchers should take the opportunity to avoid additional chemicals in any ophthalmic formulation, if possible, unless new data suggest some previously unrecognized benefits. Moxifloxacin ophthalmic solution (VIGAMOX®, Alcon Laboratories, Inc., Fort Worth, TX) is the first fluoroquinolone antibiotic preparation available in the US that does not need benzalkonium chloride to be adequately preserved (F4). There are a number of benzalkonium chloride–free fluoroquinolone products for ophthalmology available in Japan.

F1 Alfonso EC: Why is the next generation of antibiotics so important? Refractive Eyecare Ophthalmol 7(Suppl):1, 2003.

F2 AAO, Cornea/External Disease Panel and the Preferred Practice Patterns Committee: Conjunctivitis. San Francisco, AAO, 2003.

F3 Tepedino ME: Microbiological analyses of the activity of 7 anti-infectives against isolates from patients with acute bacterial conjunctivitis (abstract). Invest Ophthalmol Vis Sci 45(Suppl): 4914, 2004.

F4 Schlech BA, Sutton S, Rosenthal RA, et al: Antimicrobial preservative effectiveness of VIGAMOX®™ (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4913, 2004.

Therapeutic Usage

Topical therapy for treatment of infections remains an important and convenient avenue for the physician. The ability of an antibiotic to cure an ocular infection quickly and prevent serious vision loss is a paramount consideration for evaluating the effectiveness of therapeutic antibiotics. The ability of the antibiotic to penetrate the ocular tissues and kill and eradicate the pathogens at the site of the infection is an important goal. At this time, antibiotics such as moxifloxacin have better ocular penetration qualities than earlier fluoroquinolones, such as ciprofloxacin or ofloxacin (F5, F6, F7).[15,22]

Prophylactic Usage

The prevention of infections before, during, and after surgery and the use of prophylactic antibiotic products will undoubtedly continue in the future.[1,7,20,21,25] With this use, the antibiotic with the widest spectrum, lowest number of resistant strains, and fewest side effects should be the agent of choice for prophylaxis. The broad, shotgun approach still has merit in the surgical suite (F8).[10,17,23,25]

Antibiotic Susceptibility Testing and Breakpoints

The future of susceptibility testing is uncertain. The links between tests that define a pathogen as resistant or susceptible to a particular antibiotic are coming under fire. Standards have been set for systemic breakpoints for most antibiotics, but it is argued that these levels are not really relevant to the antibiotic levels achievable in ocular tissues via topical dosing.[19] The relatively poor predictive value of in vitro susceptibility is even dramatized more when systemic breakpoints are applied to ophthalmic antibiotics and

their usage.[26] However, MICs are still of great value for rank ordering the power of various antibiotics or comparing the organisms that make up the most resistant or less sensitive groups.[5]

Culturing Bacterial Pathogens

Isolating and identifying an infecting organism is still a key principle of the 1883–1884 postulates defined by Robert Koch. Microbiologists will continue to show the virtue of culturing, isolating, or detecting the main pathogen in ophthalmic infections. It has been shown that culture confirmation affects the antibacterial therapeutic response rate of ulcerative keratitis.[28] Corneal infections by relatively ciprofloxacin-resistant bacteria respond more slowly to ciprofloxacin therapy. Antibacterial susceptibility testing of corneal cultures may predict the fluoroquinolone therapeutic response rate of bacterial keratitis.[27]

Ideal Antibiotic Product for Ophthalmology

For today's time and considering all of the characteristics for an ideal ophthalmic antibiotic product, moxifloxacin ophthalmic solution 0.5% (as VIGA-MOX®) represents a primary antibiotic product of choice for treating and preventing ophthalmic infections. This includes improved effectiveness and potency, spectrum breadth, utility in treating and preventing infections, greater solubility, enhanced ocular penetration, acceptable safety, lack of benzalkonium chloride, and lower risk of resistance development. These virtues have been highlighted in this supplement. Nevertheless, the future will require even more advanced medications and therapy options. Such is the nature of infectious disease control for ophthalmology.

Method of Literature Search

We performed a literature search for this article based on MEDLINE database searches from 1990 to 2005, using varying combinations of the search terms *ocular infections, ophthalmic antibiotics, moxifloxacin, gatifloxacin, ciprofloxacin, ofloxacin, fluoroquinolones, therapy, prophylaxis,* and *future.* Relevant English journal articles and/or abstracts were selected for review.[6]

F5 Robertson SM, Sanders M, Jasheway D, et al: Absorption and distribution of moxifloxacin, ofloxacin and gatifloxacin into ocular tissues and plasma following topical ocular administration to pigmented rabbits (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4906, 2004.

F6 Robertson SM, Sanders M, Jasheway D, et al: Penetration and distribution of moxifloxacin and ofloxacin into ocular tissues and plasma following topical ocular administration to pigmented rabbits (abstract). Invest Ophthalmol Vis Sci 44(Suppl):1454, 2003.

F7 Solomon R, Donnenfeld E, Perry H, et al: Penetration of topically applied gatifloxacin 0.3%, moxifloxacin 0.5%, and ciprofloxacin 0.3% into the aqueous humor (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4928, 2004.

F8 Shah M, Ritterband D, Dingley A, et al: Will the new generation of fluoroquinolone antibiotics become the antimicrobials of choice in endophthalmitis prophylaxis? Abstract 12, Presented at the meeting of the Ocular Microbiology Immunology Group, 2002.

References

1. Abshire R, Cockrum P, Crider J, Schlech B: Topical antibacterial therapy for mycobacterial keratitis: potential for surgical prophylaxis and treatment. Clin Ther 26:191–7, 2004
2. Alexandrakis G, Alfonso EC, Miller D: Shifting trends in bacterial keratitis in South Florida and emerging resistance to fluoroquinolones. Ophthalmology 107:1497–502, 2000

3. Aliprandis E, Ciralsky J, Lai H, et al: Comparative efficacy of topical moxifloxacin versus ciprofloxacin and vancomycin in the treatment of *P. aeruginosa* and ciprofloxacin-resistant MRSA keratitis in rabbits. Cornea 24:201–5, 2005

4. Blondeau JM: Fluoroquinolones: mechanism of action, classification, and development of resistance. Surv Ophthalmol 49(Suppl 2):S73–S78, 2004

5. Blondeau JM: A review of the comparative *in-vitro* activities of 12 antimicrobial agents, with a focus on five new "respiratory quinolone". J Antimicrob Chemother 43(Suppl B):1–11, 1999

6. Blondeau JM, Hansen G, Metzler K, Hedlin P: The role of PK/PD parameters to avoid selection and increase of resistance: mutant prevention concentration. J Chemother 16(Suppl 3):1–19, 2004

7. Bratzler DW, Houck PM: Surgical Infection Prevention Guidelines Writers Workgroup: Antimicrobial prophylaxis for surgery: an advisory statement from the National Surgical Infection Prevention Project. Clin Infect Dis 38:1706–15, 2004

8. Chou TM, Gigliotti F, Lichtenstein SJ: Bacterial conjunctivitis: setting a course for containment and cure. Contemp Pediatr 49:1–8, 2004

9. Dajcs J, Moreau J, Thibodeaux BA, et al: Effectiveness of ciprofloxacin and ofloxacin in a prophylaxis model of *Staphylococcus* keratitis. Cornea 20:878–80, 2001

10. Dajcs JJ, Thibodeaux BA, Marquart ME, et al: Effectiveness of ciprofloxacin, levofloxacin, or moxifloxacin for treatment of experimental *Staphylococcus aureus* keratitis. Antimicrob Agents Chemother 48:1948–52, 2004

11. Dalhoff A, Schmitz FJ: *In vitro* antibacterial activity and pharmacodynamics of new quinolones. Eur J Clin Microbiol Infect Dis 22:203–21, 2003

12. Foulks G: Bacterial infections of the conjunctiva and cornea, in Albert DM, Jakobiec FA (eds): Principles and Practice of Ophthalmology VI, volume 1: Philadelphia, WB Saunders; 1994, pp.162–71

13. Goldstein MH, Kowalski RP, Gordon YJ, Baum J: Emerging fluoroquinolone resistance in bacterial keratitis: a 5-year review. Ophthalmology 106:1313–8, 1999

14. Gutierrez EH: Bacterial infections of the eye, in Locatcher-Khorazo D, Seegal BC (eds): Microbiology of the Eye. St.Louis, CV Mosby, 1972, pp. 63–76

15. Hariprasad SM, Blinder KJ, Shah GK, et al: Penetration pharmacokinetics of topically administered 0.5% moxifloxacin ophthalmic solution in human aqueous and vitreous. Arch Ophthalmol 123:39–43, 2005

16. Hwang DG: Fluoroquinolone resistance in ophthalmology and the potential role for newer ophthalmic fluoroquinolones. Surv Ophthalmol 49(Suppl 2):S79–S83, 2004

17. Kowalski RP, Romanowski EG, Mah FS, et al: Topical prophylaxis with moxifloxacin prevents endophthalmitis in a rabbit model. Am J Ophthalmol 138:33–7, 2004

18. Lohr JA: Treatment of conjunctivitis in infants and children. Pediatr Ann 22:359–64, 1993

19. NCCLS : National Committee for Clinical Laboratory Standards: performance standards for antimicrobial susceptibility testing; twelfth informational supplement. Document M100–S12. Wayne, NCCLS, 2002

20. Olson RJ: Challenges in ocular infectious diseases and the evolution of anti-infective therapy. Surv Ophthalmol 49(Suppl 2):S53–S54, 2004

21. Olson RJ: Reducing the risk of postoperative endophthalmitis. Surv Ophthalmol 49(Suppl 2):S55–S61, 2004

22. Robertson SM, Curtis MA, Schlech BA, et al: Ocular pharmacokinetics of moxifloxacin after topical treatment of animals and humans. Surv Ophthalmol 50(Suppl 1):xxx–xxx, 2005

23. Thibodeaux BA, Dajcs JJ, Caballero AR, et al: Quantitative comparison of fluoroquinolone therapies of exerimental gram-negative bacterial keratitis. Curr Eye Res 28:337–42, 2004

24. Thomson CJ: The global epidemiology of resistance of ciprofloxacin and the changing nature of antibiotic resistance: a 10 year perspective. J Antimicrob Chemother 43(Suppl A): 31–40, 1999

25. Tipperman R: Pharmacologic considerations for cataract surgery. Curr Opin Ophthalmol 15:51–5, 2004

26. Varaldo PE: Antimicrobial resistance and susceptibility testing: an evergreen topic. J Antimicrob Chemother 50:1–4, 2002

27. Wilhelmus KR, Abshire RL, Schlech BA: Influence of fluoroquinolone susceptibility on the therapeutic response of fluoroquinolone-treated bacterial keratitis. Arch Ophthalmol 121:1229–33, 2003

28. Wilhelmus KR, Schlech BA: Clinical and epidemiological advantages of culturing bacterial keratitis. Cornea 23:38–42, 2004

(4) R. Braithwaite, S. Montgomery, and S. Dawling, *Clin. Pharmacol. Ther.* **23**, 303 (1978).

(5) S. A. Montgomery, R. McAuley, D. B. Montgomery, R. A. Braithwaite, and S. Dawling, *Clin. Pharmacokinet.*, **4**, 129 (1979).

(6) D. J. Brunswick, J. D. Amsterdam, J. Mendels, and S. L. Stern, *Clin. Pharmacol. Ther.*, **25**, 605 (1979).

(7) J. R. Koup, C. M. Sack, A. L. Smith, and M. Gibaldi, *Clin. Pharmacokinet.*, **4**, 460 (1979).

(8) J. R. Koup, C. M. Sack, A. L. Smith, N. N. Neely, and M. Gibaldi, *Clin. Pharmacokinet.*, **6**, 83 (1981).

(9) G. G. Shapiro, J. R. Koup, C. T. Furukawa, W. E. Pierson, M. Gibaldi, D. Futuay, and C. W. Bierman, *Pediatrics*, **69**, 70 (1982).

(10) J. T. Slattery, M. Gibaldi, J. R. Koup, *Clin. Pharmacokinet.*, **5**, 377 (1980).

(11) J. T. Slattery, *J. Pharm. Sci.*, **70**, 1174 (1981).

(12) "Pharmacokinetics," M. Gibaldi and D. Perrier Eds, Dekker, New York, N.Y., 1975, pp. 48–60.

(13) A. Amidsen, *Dan. Med. Bull.*, **22**, 277 (1975).

(14) F. Nielsen-Kudsk and A. Amdisen, *Eur. J. Clin. Pharmacol.*, **16**, 271 (1979).

(15) "Applied Regression Analysis and Other Multivariable Methods," D. G. Kleinbaum and L. L. Kupper, Eds., Duxbury Press, North Scituate, Mass., 1978, pp. 37–50.

(16) J. M. Wilson and J. T. Slattery, *J. Pharm. Sci.*, **72**, in press.

(17) L. B. Scheiner, S. Beal, B. Rosenberg, and V. V. Marathe, *Clin. Pharmacol. Ther.*, **26**, 294 (1979).

(18) F. Gengo, J. Timko, J. D'Antonio, T. A. Ramsey, A. Frazer, and J. Mendels, *J. Clin. Psych.*, **41**, 319 (1980).

(19) J. H. Oppenheimer, H. L. Schwartz, and M. I. Surks, *J. Clin. Endocrin. Metab.*, **41**, 319 (1975).

Corneal Penetration Behavior of β-Blocking Agents I: Physicochemical Factors

RONALD D. SCHOENWALD * and HONG-SHIAN HUANG *

Abstract □ Rabbit corneas were excised and mounted in a chamber to determine the permeability characteristics of a group of β-blocking agents which varied in octanol–water partitioning over a fourfold logarithmic range. From the permeability rate at steady state, permeability coefficients (pH 7.65) were determined. For each drug the distribution coefficient and pK_a were measured, permitting the partition coefficient to be estimated. Various correlations were determined for the log permeability coefficient as a sum of log functions of the partition (or distribution) coefficient, molecular weight, and/or degree of ionization. The best fit, as judged by a high correlation coefficient ($r = 0.9756$) and lack of systematic deviation, was represented by: log $P_T = 0.623$ log DC − 0.108(log DC)^2 − 5.0268.

Keyphrases □ β-Blocking agents—permeability characteristics, excised rabbit corneas, physicochemical factors □ Permeability—β-blocking agents, excised rabbit corneas, physicochemical factors □ Ophthalmic drugs—β-blocking agents, corneal permeability, rabbits, physicochemical factors

Whenever an ophthalmic drug is applied topically to the eye, only a small amount (<10%) actually penetrates the cornea and reaches the internal eye tissues (1–3). Precorneal factors, such as rapid drainage by the nasolacrimal apparatus and noncorneal absorption, account for the poor absorption (4). As a result, optimal absorption depends on achieving a rapid penetration rate across the cornea to minimize the competing, but nonabsorptive rate factors. Rapid penetration either permits a lower dose to be administered or, in the case of an inactive drug, leads to the development of a clinically effective drug.

The penetration potential of a drug with regard to its chemical structure can be assessed by the use of the partition coefficient of the drug. This has been shown for the cornea by Schoenwald and Ward (5) and by Mosher and Mikkelson (6). Schoenwald and Ward (5) determined the permeability rates across excised rabbit corneas for 11 steroids. Permeability coefficients for each steroid were calculated, and their logarithms were plotted against their respective log octanol–water partition coefficients. A

parabolic relationship fit the data, with optimal permeability observed at a log partition coefficient of 2.9. Likewise, Mosher and Mikkelson (6) determined the *in vitro* corneal transport of *n*-alkyl-*p*-aminobenzoate ester homologues. For this series a parabolic equation also fit the data; optimal permeability was observed at a log partition coefficient of 2.5–2.6 (*n*-propyl homologue).

Although relative potency is a significant factor, a rapid penetration rate can contribute significantly to effectiveness. For example, prednisolone acetate (1% ophthalmic suspension) has been ranked as the most effective topical anti-inflammatory agent when the epithelium of the inflamed cornea is intact (7), whereas prednisolone (equally potent orally) is not effective topically. The prodrug di-
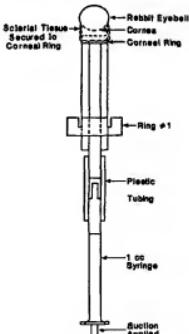
Figure 1—*Corneal holder for excised corneal preparation used in the permeability experiment.*

APPENDIX F

Table I—Chemical Structures, pKₐ, and Partition Coefficients of β-Blocking Agents
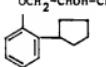
Structure	Compound	pKₐ	PCᵃ
Very lipophilic:			
OCH₂-CHOH-CH₂-NH-C(CH₃)₃	Penbut-olol	9.26	14,200.0
CHOH-CH₂-NH-C(CH₃)₃ (benzofuran, CH₂CH₃)	Bufura-lol	8.97	4,460.0
CHOH-CH₂NH-CH₂CH₂— (OCH₃, OCH₃, CH₂, O, CH₃)	Bevant-olol	8.38	1010.0
OCH₂-CHOH-CH₂-NH-CH(CH₃)₂ (naphthalene)	Propra-nolol	9.23	1640.0
Lipophilic:			
OCH₂-CHOH-CH₂-NH-C(CH₃)₃ (tetralin, O)	Levbu-nolol	9.32	249.0
O-CH₂-CHOH-CH₂-NH-CH(CH₃)₂ (OCH₂-CH=CH₂)	Oxpren-olol	9.32	235.0
CH₂-CHOH-CH₂-NH-C(CH₃)₃ (thiadiazole, morpholine, O)	Timolol	9.21	82.0
OCH₂-CHOH-CH₂-NH-CH(CH₃)₂ (CH₂CH₂OCH₃)	Metop-rolol	9.24	76.0
Hydrophilic:			
O-CH₂-CHOH-CH₂-NH-CH(CH₃)₂ (COCH₃, NHOC-CH₂CH₂CH₃)	Acebut-olol	9.20	59.0
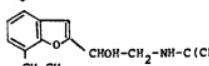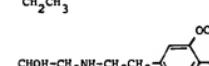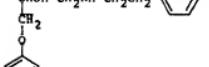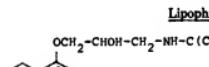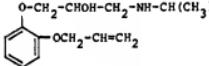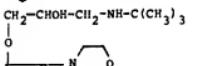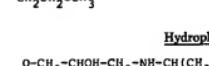
continued

Table I—Continued
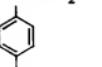
Structure	Compound	pKₐ	PCᵃ
Hydrophilic:			
CHOH-CH₂-NH-CH(CH₃)₂ (NHSO₂CH₃)	Sotalol	pKₐ₁ = 8.15 pKₐ₂ = 9.65	0.24
OCH₂-CHOH-CH₂-NH-C(CH₃)₃ (decalin, OH, H, H, OH)	Nadolol	9.39	8.5
OCH₂-CHOH-CH₂-NH-CH(CH₃)₂ (CH₂CONH₂)	Atenol-ol	9.32	1.46
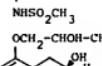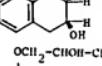
ᵃ Octanol–aqueous partition coefficient; the distribution coefficient was determined at pH 7.4 and 35° and converted through Eq. 2 to PC.

pivefrin is another example of a drug with improved corneal penetration when compared with the parent drug, epinephrine (8). A more rapid penetration rate for the prodrug has led to use of a reduced dosage and the observation of less ocular side effects.

The β-blocking agent timolol was introduced commercially to treat glaucoma following topical instillation of eye drops. Propranolol (9), atenolol (10), metoprolol (11), and practolol (10) also lower intraocular pressure, whereas nadolol and sotalol appear not to (12), even though nadolol is approximately equal in potency to propranolol. The purpose of this study was to compare the permeability of a series of β-blocking agents with a fourfold range in partitioning behavior across excised rabbit corneas to determine if optimal permeability can be identified.

EXPERIMENTAL

Drugs—β-Blocking agents used in the experiments included acebutolol hydrochloride[1], atenolol[2], beventolol hydrochloride[3], bufuralol hydrochloride[4], levbunolol hydrochloride[5], metoprolol tartrate[5], nadolol[6], oxprenolol hydrochloride[6], penbutolol sulfate[7], propranolol hydrochloride[8], sotalol hydrochloride[9], and timolol maleate[10]. Structures of each drug are shown in Table I.

Potentiometric Titration Method for the Determination of pKₐ—A pH meter[11] connected to a titrator[12] and equipped with a combination electrode[13] was used. The titrator was equipped with a 1.0-ml, syringe-type buret and was used for all titrations in the study. The buret was attached to a delivery tip capable of accurately metering the 0.005

[1] May & Baker LTD Research Laboratories.
[2] Stuart Pharmaceuticals, Division of ICI Americas Inc., Wilmington, Del.
[3] Warner-Lambert Company, Pharmaceutical Research Division, Ann Arbor, Mich.
[4] Roche Products LTD, Research Department.
[5] CIBA Pharmaceutical Co., Division of CIBA-GEIGY Corp., Summit, N.J.
[6] E. R. Squibb & Sons, Inc., Princeton, N.J.
[7] Hoechst-Roussel Pharmaceuticals. Inc., Somerville, N.J.
[8] Ayerst Laboratories, Inc., New York, N.Y.
[9] Mead Johnson & Company, Evansville, Ind.
[10] Merck Sharp & Dohme Research Lab, Division of Merck & Co., Inc., Rahway, N.J.
[11] Model 620, Fisher Accumet pH meter.
[12] Metrohm Multi-Dosimat E415, Herisau, Switzerland.
[13] Metrohm AG 9100, Herisau, Switzerland.

Journal of Pharmaceutical Sciences / **1257**
Vol. 72, No. 11, November 1983
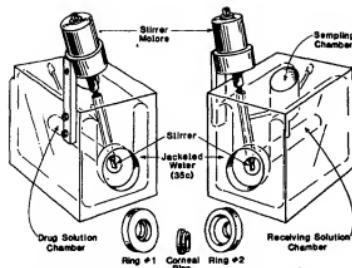
Figure 2—*Modified perfusion chamber with the installation of two stirrers; the corneal rings and chambers are also pictured.*

ml of titrant into the titration cell. The cell had a capacity of 50 ml and was surrounded by a jacket through which 35° water was circulating. A 0.29-mg/ml solution (1 mM) of a salt of each β-blocking agent was prepared for titration. In the event that the drug species was a free base, an equivalent amount of hydrochloric acid was added. For some highly lipophilic drugs, such as penbutolol and bufuralol, a concentration as low as 100 μg/ml was used to prevent precipitation during drug titration.

An aliquot (25–40 ml) of drug solution was accurately transferred to the titration cell, maintained at 35°. Nitrogen continuously flowed over the sample solution to prevent carbon dioxide absorption from the surrounding air. At each titration interval, a volume of 0.005–0.02 ml of titrant was added while stirring. For the majority of β-blocking agents, which have pK_a values of 8–9.4 at 35°, the titration usually started at pH ~6 and ended at pH 10 or 11. A modified Gran plot was used to determine the K_a for each compound (13) with the exception of sotalol, which contained two K_a values determined by the Speakman method (14).

Determination of Distribution Coefficients (15)—Sorensen's phosphate buffer (pH 7.26) was prepared from monobasic potassium phosphate and dibasic sodium phosphate. The buffer and octanol were mutually saturated at 35° before use. The distribution coefficient at 35° was determined by dissolving drug in the aqueous-buffer phase and shaking intermittently with octanol at 35° for 5 hr to reach a distribution equilibrium. The volume ratio of octanol and buffer depended on the lipophilicity of the drug. The volumes of each phase were chosen so that drug concentration in the aqueous phase, before and after extraction, could be measured by high-performance liquid chromatography (HPLC). Centrifugation was used to separate the two phases.

The distribution coefficient (DC) was calculated by:

$$DC = \frac{(C_b - C_a)V_w}{C_b V_o} \qquad (Eq. 1)$$

where C_b and C_a represent the concentrations in the aqueous-buffer phase before and after distribution, respectively; V_w represents the volume of the aqueous phase; and V_o, the volume of the octanol phase. The partition coefficient (PC) was calculated from the distribution coefficient by:

$$PC = DC \left(1 + \frac{1}{\text{antilog (pH} - pK_a)}\right) \qquad (Eq. 2)$$

The pH was measured from the buffered phase at 35° after distribution was complete. All distribution coefficients reported here were measured at pH 7.4, but through the use of Eq. 2 were converted to pH 7.65, the pH of the excised corneal experiments.

Excised Cornea Procedure—Male New Zealand White rabbits[14], weighing 1.6–2.0 kg each, were sacrificed by injecting a bolus of air into the marginal ear vein. The intact eye, along with the lids and conjunctival sac, was then enucleated. The exposed cornea of the enucleated eye was carefully placed on a corneal holder, which maintained the cornea curvature and held the eye in place (5, 16, 17). Various tissues of the eye were dissected leaving the cornea, a small ring of scleral tissue, and the palpebral conjunctiva, which was tied to the corneal ring (Fig. 1).

The conjunctival and scleral tissue served as a gasket and permitted

[14] Morrison Rabbitry, West Branch, Iowa.

Table II—Experimental Conditions for the HPLC Assay of β-Blocking Agents

Drug	Column[a]	Wavelength, nm	Methanol, %[b]	Flow Rate, ml/min
Acebutolol	A	254	20, 38	2
Atenolol	A	254	20, 7.5	2
Bevantolol	B	254	28, 30	2
Bufuralol	B	254	28, 30	2
Levbunolol	B	254	28, 47	2
Metoprolol	A	280	35, 35	2
Nadolol	A	254	20, 31	2
Oxprenolol	B	280	22, 15	2.5
Penbutolol	B	254	25, 62.2	2
Propranolol	B	254	28, 23	2
Sotalol	A	254	42[c], 30	2.5
Timolol	A	254	42[c], 30	2.5

[a] (A) μ-Bondapak C18; (B) μ-Bondapak CN. Waters Associates, Milford, Mass.
[b] The two numbers represent the percentage of methanol in the mobile phase for partitioning and corneal permeability determinations, respectively; the aqueous phase contained 1.5% acetic acid and was adjusted to pH 4 by sodium hydroxide.
[c] The aqueous phase consisted of 58% 0.005 M heptanesulfonic acid and 1% acetic acid; the flow rate was 2.0 ml/min for these conditions.

the cornea to be suspended within the corneal ring, which was then positioned between rings 1 and 2 and placed in the center of the perfusion chamber. The chamber was made from acrylic plastic[15] and was jacketed to maintain the cornea and the perfusion solution at 35° (5, 16, 17).

Bicarbonated Ringer's solution was modified (17) to preserve tissue integrity of an excised cornea over 6 hr and used throughout the perfusion studies. It was prepared in two parts: Part I was composed of sodium chloride (12.4 g/liter), potassium chloride (0.716 g/liter), monobasic sodium phosphate monohydrate (0.206 g/liter), and sodium bicarbonate (4.908 g/liter) and Part II was composed of calcium chloride dihydrate (0.230 g/liter), magnesium chloride hexahydrate (0.318 g/liter), glucose (1.80 g/liter), and oxidized glutathione[16] (0.184 g/liter). Both parts were stored in the refrigerator and were used in ~3 weeks to prevent mold growth. Equal volumes of parts I and II were mixed prior to use.

Within 20–40 min of death, the cornea was mounted and clamped between two cylindrical compartments of the perfusion chamber. A measured volume (7.0 ml) of bicarbonated Ringer's solution was added first to the endothelial side as the receiving solution to prevent the cornea from buckling. An equal volume of solution containing a β-blocking agent was then added to the epithelial side as the drug solution. The perfusion chamber system was designed in such a way that the height of the receiving solution was slightly higher than that of the drug solution to ensure that the cornea would not buckle during the course of the experiment. A mixture of O_2–CO_2 (95:5) was bubbled through the fluids in both chambers for 10 min to achieve a pH of 7.65 before being added to the perfusion chamber. Circulation of fluid inside each half chamber was induced immediately by bubbling the same gas mixture through at a rate of three to five bubbles/sec to maintain the solution at a constant pH of 7.65.

Samples ranged from 0.1 to 0.5 ml depending on the assay sensitivity of each drug. Samples were withdrawn from the receiving chamber (i.e., endothelial side) over a 4-hr period. An equal volume of solution was immediately added to the receiving solution to maintain a constant volume. The first sample was withdrawn within 2 min after starting the permeation and served as a control to detect leakage and rapid penetration. Subsequent samples were taken approximately every 40 min through the 4-hr period.

The sampling method for the corneal permeability experiments of levbunolol and nadolol varied from other drugs in that equal volumes of solutions (0.1 ml) were removed from both sides of cornea. In this way, equal volumes on both sides were maintained throughout the experiment.

For each permeability experiment, the cornea was trimmed of excess scleral tissue and conjunctiva, weighed, and dried in an oven overnight at 103°. After each cornea was dried, it was reweighed so that the hydration level of the wet cornea could be determined. The normal cornea has a hydration level of 76–80% (18). If manipulation of the cornea or if the drug itself led to damage of the epithelium and/or endothelium, then the hydration level would rise (83–92%) and the data were discarded.

Determination of Aqueous Diffusional Layer Resistance in the Perfusion Chamber—Fluid circulation in the chamber was provided

[15] Medical Research Instruments, University of Iowa, Iowa City, Iowa.
[16] Aldrich Chemical Co., Inc., Milwaukee, Wis.
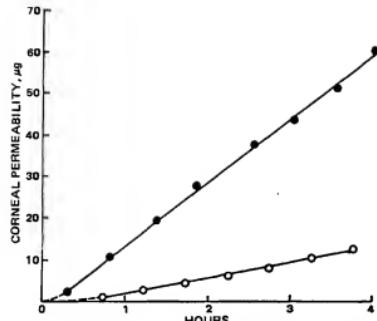
Figure 3—*Permeability rate of propranolol (●) and atenolol (○) across single excised rabbit corneas including linear regression lines; the initial concentrations for propranolol and atenolol were 85 and 2000 μg/ml, respectively.*

Figure 4—*Nonlinear regression results of the diffusional resistance of atenolol to stirring rate through excised rabbit stromal preparations. Each point is the average of 3–6 determinations; standard deviations were ≤10% of the mean.*

by maintaining a rate of three to five gas bubbles/sec. This maintains good mixing within each half chamber, as can be shown by adding a drop of colored solution into either half of the chamber and observing the homogeneous color that occurs in <1 min. However, to accurately determine the resistance of the cornea, it was necessary to detect and measure the magnitude of aqueous diffusional layer resistance, R_{aq}; consequently, the stirring was modified for these experiments.

The perfusion chamber was modified by installing two stirrers, one on each side of the cornea, with the center of each stirrer affixed 1 cm from the center of the corneal ring. Figure 2 depicts the modified perfusion chamber and rings used in mounting the cornea. In preliminary experiments it was observed that different rates of stirring induced varying degrees of swelling due to mechanical injury of the epithelium and endothelium.

An important purpose of the epithelium and endothelium is to control the thickness of the cornea by maintaining hydration levels at ~78%. By completely removing the epithelium and endothelium, the remaining stromal layer reached a constant and maximal thickness within the first 30 min of stirring such that subsequent changes in the stirring rate had no effect. The epithelium was removed by scraping with the blunt end of a scalpel blade. The endothelium was gently rubbed off with a cotton-tipped applicator (19). The removal of endothelium could be detected with the aid of a dissecting microscope. The epithelium was removed immediately following enucleation; the endothelium was removed just prior to mounting in the perfusion chamber. Atenolol (500 μg/ml) was chosen as the diffusing substance for these experiments. Since the aqueous diffusional barrier is independent of drug, these results were interpreted for the other β-blocking agents as well.

Once drug was placed adjacent to the cornea, the stirring speed was increased in steps every 30 min over a 4-hr period. The apparent permeability coefficients were determined for each 30-min increment. A sample was removed for atenolol analysis at the beginning and end of each 30-min period; both samples were used to calculate the permeability coefficient for each stirring speed. To minimize biological variability, each cornea was used to generate five or six permeability coefficients over a period of 4 hr.

Drug Assay—An HPLC method was used for analysis of each drug. The HPLC method included an injector[17], solvent delivery system, UV-absorption detector[17], column[18], and recorder[19]. The injector was equipped with different-sized loops, ranging from 50 μl to 200 ml, which enabled the injection of an accurate volume of sample solution. A solution of known concentration was used as an external standard. Each sample solution was divided so that two injections could be made and the results averaged.

[17] Model 7125 injector; Rheodyne, Cotati, CA 94928.
[18] M-6000 A solvent delivery system, Model 440 absorbance detector, μ-Bondapak C18, and μ-Bondapak CN Columns; Waters Associates, Milford, MA 01757.
[19] Model 5211-1; OmniScribe; Houston Instruments, Austin, Tex.
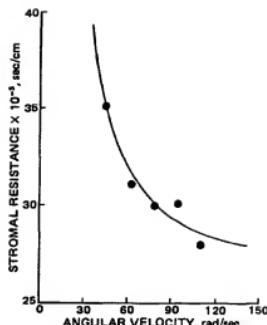
by determining the pore radius of the porous barrier. The mobile phase consisted of varying ratios of methanol and deaerated, deionized water containing 1.5% acetic acid, adjusted to pH 4 using sodium hydroxide. One exception was the mobile phase for sotalol and timolol, which utilized 0.005 M heptanesulfonic acid (to increase retention time) and 1% acetic acid. Table II lists the types of columns used, wavelengths at which UV measurements were made, methanol content of the mobile phase, and mobile phase flow rate. Methanol percentages in the mobile phases varied depending on whether partitioning or permeability experiments had been conducted. Drug solutions withdrawn from the endothelial side following corneal permeability contain polar extracts from the cornea which were eluted from the column within 1–3 min. In all experiments the retention times for the drugs were between 4 and 12 min. Linearity existed over the concentrations employed for each β-blocking agent (correlation coefficient >0.99).

Calculation of Permeability Coefficients—The apparent permeability coefficient (P_{app}, cm/sec) was determined by (19):

$$P_{app} = \frac{\Delta Q}{\Delta t (3600) A C_0} \qquad (\text{Eq. 3})$$

where the term $\Delta Q/\Delta t$ is the permeability rate (*i.e.*, steady-state flux, μg/hr) of drug across each excised cornea, C_0 is the initial drug concentration (μg/ml), A is the corneal surface area (cm²), and 3600 is the conversion of hours to seconds. Corrections of C_0 were made to account for the sample volume removed over time and subsequently replaced with blank solution.

The corneal thickness increases with hydration, and the permeability coefficient is inversely proportional to barrier thickness. Therefore, it was important to determine corneal thicknesses. For a 2-kg rabbit, the corneal thickness (h) can be determined by:

$$h \ (\text{cm}) = \frac{0.42 + H}{100} \qquad (\text{Eq. 4})$$

where H is the mg of water/mg of dry tissue (20). When stromal thicknesses were swollen due to epithelial and endothelial removal, the stroma resistances, $R_{str,swl}$, that were used to assess the aqueous diffusional barrier were corrected to the normal stromal thickness as existing in intact corneas (*i.e.*, $R_{str,int}$) by:

$$R_{str,int} = R_{str,swl} \left[\frac{h_{int}}{h_{swl}} \right] \qquad (\text{Eq. 5})$$

where subscripts int and swl represent intact cornea and swollen stroma, respectively.

RESULTS AND DISCUSSION

The pK_a and partition coefficients of each β-blocking agent are listed in Table I. Although the aromatic substituents varied substantially for the series, these were too far removed from the amino group to exert much

of an effect on the pK_a values. Therefore, most pK_a values were within a narrow range (8.97–9.65). The pK_a of bevantolol was slightly lower (8.38) because of the electron-withdrawing effect of the ethoxybenzyl substituent. Sotalol has two pK_a values, 8.15 and 9.72, which are close to one another and compare reasonably well with the values of 8.30 and 9.80 published by Garrett and Schnelle (21) using the potentiometric titration method at 25°. The anilino group in sotalol acts as a weak acid as a result of the electron-withdrawing effect of the neighboring sulfonyl group. Ionization of the anilino group accounts for spectral shifts (21) and correlates with the pK_a of 8.15. The second pK_a, 9.72, was then assigned to the protonated amine group in the alkyl side chain of sotalol.

The partition coefficients obtained from the extraction method using octanol and Sorenson's buffer varied over a fourfold log range. The range in partitioning behavior of the series is a consequence of the differences in aromatic substitution. The partitioning results permitted the compounds to be grouped as very lipophilic, lipophilic, or hydrophilic, classifications which were predictable from structural considerations. In addition to the amino group, the hydrophilic compounds also contained relatively polar substituents on the aromatic ring. Therefore, hydrogen bonding interactions with water are greater for atenolol and acebutolol, which contain amido groups, for nadolol, which contains a dihydroxy function and for sotalol, which contains a sulfonamido moiety. The very lipophilic compounds, on the other hand, contain hydrophobic substituents. The cyclopentyl group on the benzene ring gives penbutolol a high distribution and partition coefficient, whereas the furanyl group imparts lipophilicity to bufuralol. The ethoxybenzyl substituent in bevantolol not only lowers its pK_a, but also increases its lipophilicity. The high partition coefficient of propranolol is a result of the high lipophilic contribution of naphthalene. Based on the partitioning results of the very lipophilic and hydrophilic compounds, the remaining compounds (levbunolol, oxyprenolol, timolol, and metoprolol) appear to contain substituents of an intermediate nature as far as polarity.

Corneal Permeability—The permeability coefficients of each β-blocking agent were obtained by linear regression of the steady-state flux. Figure 3 shows a plot of Q versus t for propranolol and atenolol across excised rabbit corneas. The data points closely fit the least-square regression line once steady state has been reached. The lag time, defined by the linear intercept on the time axis, is related to the time required to reach steady-state permeation; more specifically, it is inversely related to the permeability coefficient. Consequently, the more rapidly penetrating compounds will have a shorter lag time and a greater steady-state flux. The slope of the straight line $(\Delta Q/\Delta t)$ was substituted into Eq. 3 to obtain the apparent permeability coefficient. The apparent permeability coefficient also contains any aqueous diffusional layers that may exist on each side of the cornea.

Mathematical Model Relating Stirring Rate to Aqueous Diffusional Layer Resistance—The total diffusional resistance, R_{app}, through a multilayered barrier is represented by (22):

$$R_{app} = \frac{1}{P_{app}} = \sum_{i=1}^{n} R_i = \sum \frac{h_i}{D_i A (PC)_i} \qquad \text{(Eq. 6)}$$

where i represents each homogeneous layer in series, n represents the total number of barriers, h represents barrier thickness, A represents surface area, D represents the diffusion coefficient, and PC represents the partition coefficient (22). With regard to significant diffusional layers, the rabbit cornea possesses two main tissue layers: the lipophilic epithelium and endothelium, and the hydrophilic stroma. Assuming the existence of an aqueous diffusional barrier, the apparent resistance of the cornea can be considered as layers in series (23, 24) or:

$$R_{app} = \frac{1}{P_{app}} = R_{epi} + R_{str} + R_{endo} + R_{aq} \qquad \text{(Eq. 7)}$$

or

$$R_{app} = R_T + R_{aq} \qquad \text{(Eq. 8)}$$

where R_{aq} represents the sum of aqueous diffusional resistances on each side of the cornea and R_T represents the sum of the resistances of the corneal layers (epithelium, stroma, and endothelium).

According to the Nernst theory (25), there is a thin layer of static liquid of thickness h_{aq} immediately adjacent to a solid body. Outside of the static liquid layer is the well-stirred bulk solution. Experimental determinations have shown that the aqueous diffusional layer thickness, h_{aq}, can be expressed as:

$$h_{aq} = V^{-n} \qquad \text{(Eq. 9)}$$

where V is the velocity of the moving liquid. The exponent n depends

on the experimental conditions ranging from $n = 0.33$ to ≥ 1. The thickness measurements representing the diffusional layer are apparent and not real. For example, experimental measurements have shown that the liquid retains its mobility down to a distance from the solid surface smaller than h_{aq}. Despite the fact that the Nernst theory may not exactly represent the diffusional behavior at the interface of liquid and solid, it can be used empirically to calculate R_{aq} (25).

In determining the resistance of the β-blocking agents across excised rabbit corneas within the stirred perfusion chamber, the following equation, which combines Eqs. 6–9, was considered:

$$R_{app} = R_{str} + \frac{h_{aq1}}{DA(PC)} + \frac{h_{aq2}}{DA(PC)} \qquad \text{(Eq. 10)}$$

where h_{aq1} and h_{aq2} are the aqueous diffusial layer thicknesses on each side of the mounted cornea at a given stirring rate, and R_{str} represents the membrane resistance for the stroma. R_{app} is measured experimentally at a specific stirring rate, i.e., $1/P_{app}$.

By assigning $h_{aq} = h_{aq1} + h_{aq2}$ and substituting V^{-n} for h_{aq}, then Eq. 10 can be expressed as:

$$R_{app} = R_{str} + \frac{V^{-n}}{DA(PC)} \qquad \text{(Eq. 11)}$$

In the modified perfusion chamber, the stirrer is at the center of a circle 1 cm in diameter which contacts tangentially with the membrane and the perfusion chamber wall. Assuming that the liquid velocity tangential to the membrane, V, is proportional to the angular velocity of stirring, v, then Eq. 9 becomes:

$$R_{app} = R_{str} + \frac{(mv)^{-n}}{DA(PC)} \qquad \text{(Eq. 12)}$$

where m is a proportionality factor between the liquid velocity (cm/sec) and the angular velocity (rad/sec)[20]. To perform the nonlinear regression analysis the diffusion coefficient (D) was approximated by 1×10^{-5} cm²/sec, an appropriate estimate for compounds of 200–300 molecular weight (22); PC was assigned a value of 1 for the aqueous system, and A was assigned a value 1.087 cm², which represented the surface area of the cornea used throughout the study. The remaining unknown parameter values (R_T, m, and n) were determined from the nonlinear regression analysis[21]. Figure 4 shows the results for atenolol permeation through excised stromal preparations with stirring rates varying from 425 to 1050 rpm. The computer-generated parameter values substituted into Eq. 10 become:

$$R_{app} = 26.8 \times 10^3 + 100,000 \, (0.1006v)^{-1.67} \qquad \text{(Eq. 13)}$$

where 26.8×10^3 sec/cm represents the intrinsic stromal resistance, R_{str}. The apparent stromal resistance to atenolol permeation was 30.5×10^3 sec/cm. This latter value represents the experimental conditions for the perfusion chamber when stirred with the bubbling action of O_2–CO_2. The difference between the two resistances ($R_{app} - R_{str}$) is 3.7×10^3 sec/cm and represents the aqueous diffusional layer resistance, R_{aq}. This value was used in determining the intrinsic membrane resistances for the other β-blocking agents.

Permeability versus Partitioning Correlations—Figure 5 shows a plot of log P_T[22] against log PC; Table III contains the calculated parameter values. The data, although somewhat scattered, shows a plateau region for the very lipophilic compounds (propranolol, bufuralol, bevantolol, and penbutolol). The rate-determining factor responsible for the plateau region is not a result of the aqueous diffusion layer, since its contribution was subtracted from the experimentally determined permeability coefficients. The permeability rate is probably controlled by the hydrophilic stroma for these very lipophilic compounds. The relatively poor permeability shown for the hydrophilic compounds nadolol and sotalol possibly explains their poor potential for lowering intraocular pressure.

Multiple regression analyses[23] (26) were performed on the data to find the best set of parameters to describe the change in log P_T with a change in either log PC or log DC. Although the ranges in molecular weight and pK_a were relatively narrow for the β-blocking agents selected for study,

---

[20] rpm was converted to rad/sec by: rad/sec = rpm(2π)/60.
[21] Nonlinear regression was performed using the BMDP3R programs on an IBM370, at the University of Iowa Computer Center, University of Iowa, Iowa City, IA 52242.
[22] P_T represents the permeability coefficient across the intact excised rabbit cornea; $P_T = 1/R_T$.
[23] Multiple linear regression was performed using the BMDP1R, BMDP2R, and BMDP9R regression programs on an IBM370 computer.
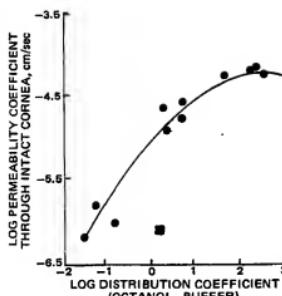
Figure 5—*Log-log plot of permeability coefficient (pH 7.65) and distribution coefficient (pH 7.65). The regression curve is represented by:* log P_T = 0.623 log DC − 0.108 (log DC)^2 − 5.0268, where r = 0.9756 and n = 11; acebutolol (■) is included in the figure, but not in the regression curve.

a log MW term and a log DI[24] term were included in the analysis. Molecular weight (MW) is inversely related to diffusion and has been shown to improve correlations of this type (27, 28). Because of the plateau region (Fig. 5), a log PC)^2 or (log DC)^2 term was also included. All possible subsets were analyzed, beginning with the intercept plus one parameter, then the intercept plus two parameters, etc., up to the single set representing the intercept plus the maximum of four parameters. The correlation coefficient (r) and systematic deviation were used as the criteria to judge the best fit. The best fit for log P_T was represented as a function of all the parameters:

$$\log P_T = 1.01 \log PC - 0.115(\log PC)^2 - 5.64 \log MW$$
$$- 10.4 \log DI + 7.27 \qquad (Eq.\ 14)$$
$$r = 0.9272 \qquad p = 0.0041 \qquad n = 12$$

Both molecular weight and degree of ionization showed the expected inverse relationship to permeability. However, with sither the molecular weight or degree of ionization term omitted from the regression analysis, the correlation coefficient was reduced only minimally to 0.8989 or 0.8678, respectively. With both molecular weight and degree of ionization removed, the correlation coefficient was 0.8560. With only the log PC term and the intercept, the regression analysis yielded a correlation coefficient of 0.8523. This latter linear regression line, however, shows systematic deviation at the plateau region and was not considered an acceptable fit to the data.

When DC was substituted for PC the multiple regression analyses produced an equally good fit:

$$\log P_T = 0.681 \log DC - 0.123(\log DC)^2 - 5.04 \log MW$$
$$- 2.64 \log DI + 7.22 \qquad (Eq.\ 15)$$
$$r = 0.9282 \qquad p = 0.0040 \qquad n = 12$$

When the degrees of ionization was removed from consideration in Eq. 15, the correlation coefficient was reduced slightly to 0.9223. The lack of improvement from considering the degree of ionization is understandable, since the distribution and permeability coefficients represent the data at the same pH. By excluding the molecular weight term and (log DC)^2, the correlation coefficient was 0.8908 illustrating the small, but necessary, contribution of the squared term when systematic deviation is considered.

The hydrophilic acebutolol deviated the most from any regression line. By considering acebutolol as an outlier and omitting it from the regression analysis, the correlation coefficients increased. For example, the best regression lines yielded:

$$\log P_T = 0.972 \log PC - 0.112(\log PC)^2 - 2.71 \log MW$$
$$- 9.26 \log DI + 0.219 \qquad (Eq.\ 16)$$

[24] DI represents degree of ionization and was calculated from: DI = 1/[1 + antilog (pH − pK_a)].

$$r = 0.9696 \qquad p = 0.0008 \qquad n = 11$$
$$\log P_T = 0.623 \log DC - 0.108(\log DC)^2 - 5.0268 \qquad (Eq.\ 17)$$
$$r = 0.9756 \qquad p < 0.00009 \qquad n = 11$$

Equation 17 predicts an optimum log DC value of 2.88, determined by setting d log P_T/d log DC equal to zero and solving for log DC. However, there is no experimental evidence that a parabola would best describe the data. Compounds of greater lipophilicity than penbutolol could not be obtained to test this phenomenon.

Although correlations of this type are helpful in predicting useful molecular modifications, extrapolation to *in vivo* ophthalmic bioavailability must take into consideration solubility, the short residence time of instilled drops in the eye, and rapid metabolism or poor distribution to the target tissue. For example, s drug may have ideal partitioning behavior, but if it is not soluble, its concentration in tears will be too low to achieve an adequate penetration rate since the penetration rate is equal to the permeability coefficient multiplied by tear concentration. If a suspension is formulated because of the poor drug solubility, expulsion of the particles by the eye may take place before solubilization occurs, resulting in lower bioavailability.

REFERENCES

(1) R. Lazare and M. Horlington, *Exp. Eye Res.*, 21, 281 (1975).
(2) T. F. Patton and J. R. Robinson, *J. Pharm. Sci.*, 65, 1295 (1976).
(3) H. Benson, *Arch Ophthalmol.*, 91, 313 (1974).
(4) V. H.-L. Lee and J. R. Robinson, *J. Pharm. Sci.*, 68, 673 (1979).
(5) R. D. Schoenwald and R. L. Ward, *J. Pharm. Sci.*, 67, 786 (1978).
(6) G. L. Mosher and T. J. Mikkelson, *Int. J. Pharm.*, 2, 239 (1979).
(7) H. M. Leibowitz and A. Kupferman, *Invest. Ophthalmol.*, 13, 757 (1974).
(8) A. I. Mandell and S. M. Podos, "Dipivalyl Epinephrine (DPE): A New Pro-Drug in the Treatment of Glaucoma," in "Symposium of Ocular Therapy," Vol. 10, Wiley, 1977.
(9) J. Vale, A. C. Gibbs, and C. I. Phillips, *Br. J. Ophthalmol.*, 56, 770 (1972).
(10) K. Wettrell, *Acta Ophthalmol., Suppl.*, 134 (1977).
(11) N. V. Neilsen, *Acta Ophthalmol.*, 59, 495 (1981).
(12) M. V. W. Bergamini, J. Anderson, V. J. Rajadhyaksha, and R. Schoenwald, "Diacetyl Nadolol and Triacetyl Nadolol: Potential Prodrugs of Nadolol with Ocular Beta Blocking Activity," presented at the Association for Research and Vision in Ophthalmology meeting, Sarasota, Fla., May 1982.
(13) F. J. C. Rossotti and H. Rossotti, *Jr. Chem. Ed.*, 42, 375 (1965).
(14) J. C. Speakman, *J. Am. Chem. Soc.*, 62, 855 (1940).
(15) C. Hansch, in "Strategy of Drug Design," Appendix I W. P. Purcell, G. E. Bass and J. M. Clayton, Eds, Wiley, New York, N.Y., 1973.
(16) H. F. Edelhauser, J. R. Hoffert and P. O. Fromm, *Invest. Ophthalmol.*, 4, 290 (1965).
(17) W. J. O'Brien and H. F. Edelhauser, *Invest. Ophthalmol. Visual Sci.*, 16, 1093 (1977).

(18) D. M. Maurice and M. V. Riley, in "Biochemistry of the Eye," C. N. Graymore, Ed., Academic, London and New York, 1970, pp. 6–16.
(19) J. H. Kim, K. Green, M. Martinez, and D. Paton, *Exp. Eye Res.*, 12, 231 (1971).
(20) B. O. Hedbys and S. Mishima, *Exp. Eye Res.*, 5, 221 (1966).
(21) E. R. Garrett and K. Schnelle, *J. Pharm. Sci.*, 60, 833 (1971).
(22) G. L. Flynn, S. H. Yalkowsky, and T. J. Roseman, *J. Pharm. Sci.*, 63, 479 (1974).
(23) I. Fatt in "Physiology of the Eye," Butterworth, Woburn, Mass., 1978, pp. 114–121.
(24) R. D. Schoenwald and J. A. Houseman, *Biopharm. Drug Dispos.*, (1982), in press.
(25) V. G. Levich, in "Physicochemical Hydrodynamics," Prentice Hall, Englewood Cliffs, N.J., 1962, pp. 40–46.
(26) N. Draper and H. Smith, in "Applied Regression Analysis," 2nd ed., Wiley, New York, N.Y., 1981, pp. 294–312.
(27) E. J. Lien and P. H. Wang, *J. Pharm. Sci.*, 69, 648 (1980).
(28) E. J. Lien, A. A. Alhaider, and V. H.-L. Lee, *Jr. Parenter. Sci. Technol.*, 36, 86 (1982).

Corneal Penetration Behavior of β-Blocking Agents II: Assessment of Barrier Contributions

HONG-SHIAN HUANG *, RONALD D. SCHOENWALD ×, and JOHN L. LACH

Abstract □ Rabbit corneas were excised and mounted in a chamber to determine the permeability characteristics of a group of β-blocking agents. By measuring the permeability rate of each drug across intact cornea, stroma alone, epithelium–stroma, and stroma–endothelium, it was possible to determine the resistance to penetration for each corneal layer. The reciprocal of the sum of resistances to penetration for the epithelium, stroma, and endothelium equaled the experimentally determined permeability coefficient for the intact cornea (104 ± 6.0%). Thus, the penetration of β-blocking agents through the excised rabbit cornea could be treated as three barriers in series. For hydrophilic compounds, the epithelium was the rate-determining barrier. The endothelium offered less resistance, whereas the stroma offered only very minimal resistance. The lipophilic compounds penetrated the excised cornea more rapidly. However, the stroma became rate-determining for the most lipophilic compounds (penbutolol, bufralol, bevantolol, and propranolol). Although the octanol–buffer (pH 7.65) distribution coefficient of these compounds varied over a fourfold logarithmic range, the permeability coefficient was considered nearly constant [3.4 × 10⁻⁵ (±0.34) cm/sec] for stroma. Also, the ratios of tortuosity to porosity for the stromal layer were 1.58 ± 0.15. These results suggest that drug diffuses through an aqueous media of gel-like mucopolysaccharide interspersed by a matrix of collagen fibrils. From further analyses intra- and intercellular pathways for epithelium and endothelium were added to the model resulting in a sigmoidal representation of permeability coefficient versus distribution coefficient. However, the intercellular (pore) pathway could not be adequately quantified because of the variation in the data for very hydrophilic compounds.

Keyphrases □ β-Blocking agents—permeability characteristics, excised rabbit corneas, barrier contributions □ Permeability—β-blocking agents, excised rabbit corneas, barrier contributions □ Ophthalmic drugs—β-blocking agents, corneal permeability, rabbits, barrier contributions

To optimize the penetration rate of drugs across biological membranes, quantitative multiple regression analyses are conducted to relate permeability to various physicochemical factors (1–3). These factors are often related through a sum of log terms, including partition coefficient, molecular weight, and degree of ionization. With the use of a digital computer and the appropriate algorithms, the regression analysis can be performed by a stepwise addition or deletion of each term or by comparing all possible subsets of the terms (4). In this way the

significance of each term can be ascertained. Once all relevant physiochemical properties have been defined, an optimal chemical structure can be proposed. This semi-empirical approach, however, does not characterize the biological limitations imposed by the membrane, such as limiting diffusional layers.

The permeability coefficients (P_T) of 12 β-blocking agents through excised rabbit corneas mounted in a perfusion chamber at pH 7.65 were determined in the previous paper (5). Through multiple regression analyses (excluding one outlier), log P_T could be related to partitioning factors by:

$$\log P_T = 0.6228 \log DC - 0.1081(\log DC)^2 - 5.03$$

$$r = 0.9756 \quad p < 0.00009 \quad n = 11 \quad \text{(Eq. 1)}$$

where DC represents the octanol–buffer (pH 7.65) distribution coefficient. Neither a log molecular weight term nor a log degree of ionization term significantly improved the correlation. The parabolic equation represented in Eq. 1 predicted optimal penetrability at a log DC value of 2.88, the apex of the parabola. However, the experimental data (log P_T versus log DC) was curvilinear, leveling off to a plateau such that the asymptotic transport model of Ho et al. (6) could be applied. It is the purpose of this study to determine the limiting biological factors governing the steady-state flux of β-blocking agents across the multi-layered excised rabbit cornea.

EXPERIMENTAL

Drugs—β-Blocking agents used in the experiments were acebutolol hydrochloride[1], atenolol[2], bevantolol hydrochloride[3], bufralol hydrochloride[4], levbunolol hydrochloride[3], metoprolol tartrate[5], nadolol[6],

Corneal Perfusion Chambers
Moxifloxacin Rate of Diffusion (Flux)

1.0%
0.75%
0.5%
0.3%
0.1%

6.00 µg/min
4.54 µg/min
3.20 µg/min
2.16 µg/min
0.86 µg/min

Time (min)

Amount (µg)

APPENDIX G1

Corneal Perfusion Chambers
Rate of Diffusion (Flux) of Moxifloxacin

Concentration of Moxifloxacin	n	Slope (µg/min)	Total Accumulation after 240 min	Diffusion Coefficient Papp (10^{-7} cm/sec)	Lag Time (min)
0.1%	2	0.86	180	145	45
0.3%	2	2.16	455	121	29
0.5%	4	3.20	667	100	32
0.75%	2	4.54	930	102	35
1.0%	2	6.00	1197	101	41

CORNEAL PENETRATION AND CHANGES IN CORNEAL PERMEABILITY OF MOXIFLOXACIN VERSUS GATIFLOXACIN

G.R. Owen, O. Dembinska, K.R. Stout, and M.K. Mendiola

Alcon Research Ltd., 6201 South Freeway, Fort Worth, Texas 76134

Abstract

Purpose: To compare the effects upon the cornea of moxifloxacin versus gatifloxacin by determining the corneal penetration and change in corneal epithelial permeability following exposure to fluoroquinolone solutions in ex vivo corneal perfusion models. Methods: The penetration studies were conducted with solutions of the fluoroquinolones (0.1mM), and the permeability studies were conducted with the commercial preparations Vigamox™ (0.5% moxifloxacin, Alcon Laboratories, Inc.) and Zymar™ (0.3% gatifloxacin, Allergan, Inc.), which contains 0.005% BAC. In both sets of experiments, corneas of New Zealand white rabbits were excised and mounted in corneal perfusion chambers according to established methods. The penetration studies were conducted with the fluoroquinolone solutions applied to the epithelial side of the cornea. The rates of accumulation of the fluoroquinolones on the endothelial side of the chamber were determined using HPLC analysis of the perfusates over 5 hours. In the permeability studies, the commercial preparations were applied to the epithelial surface for 5 min. After rinsing, corneas were exposed to carboxyfluorescein (CF) for 5 min and the permeate collected over 2 hrs. The rate of CF in the perfusate was measured by spectrophotometry. Results: Moxifloxacin showed an apparent corneal permeability coefficient of 91 x 10⁶ cm/sec, compared to 25 x 10⁶ cm/sec for gatifloxacin. The lag time for the appearance of moxifloxacin at the endothelial side of the cornea was determined to be 99 minutes for gatifloxacin. However, permeability of the cornea to CF was 2.1 µMol/min for Vigamox™ versus 3.4 µMol/min for Zymar™, with peak of accumulation of 37 µMol for Vigamox™ versus 60 µMol for Zymar™. Although the drug penetration is greater, the corneal permeability is 3.6 to 1.6 x lower after exposure to Vigamox™ compared to Zymar™, illustrating that Vigamox™ retains superior corneal integrity. This demonstrates that the increased penetration of moxifloxacin is due to the inherent characteristics of the moxifloxacin molecule, and is not due to change in the corneal epithelial intracellular tight junctions.

Introduction

Moxifloxacin and gatifloxacin are both fourth generation fluoroquinolones that have recently been developed with enhanced penetration and change in corneal epithelial permeability activity, good solubility, and are less likely to develop bacterial resistance because of their dual mode of action. The purpose of the present study is to determine as complete.

- the corneal penetration of the two fluoroquinolones using ex vivo rabbit corneal pefusion models,
- steady state conditions,
- The corneal penetration effect in both in response to the commercial preparations Vigamox™ (0.5% moxifloxacin, Alcon Laboratories, Inc.) and Zymar™ (0.3% gatifloxacin, Allergan, Inc.), applied to the epithelial side of the cornea,
- Accompanying changes in corneal epithelial permeability.

Methods

Female New Zealand Albino rabbits weighing about 2 kg were handled and housed in accordance with USDA, AAALAC and OPRR Regulations & Standards. After sacrifice, the intact eyes, should be enucleated and placed immediately and kept in fresh BSS PLUS™ Irrigating Solution saturated with O_2 (95%).

- In the penetration experiments, solutions was placed on corneas were mounted in the modified endothelial side of the cornea and 0.5 was comprised in the fluoroquinolone at a 0.1 mMolar solutions of the fluoroquinolones were prepared in the BSS solution and applied to the epithelial side of the cornea. Samples were withdrawn from the BSS containing

Results

Under steady state conditions in BSS (Figure 1 and Table 1, n = 3), moxifloxacin was found to have an apparent corneal permeability coefficient (1) of 91 x 10⁶ cm/sec, compared to 25 x 10⁶ cm/sec for gatifloxacin. The proportionally larger apparent permeability coefficient of the endothelial side of the cornea provides for a net permeation of 2.3 times the endothelial side of the cornea to 3.6 times the appearance of moxifloxacin at 2.3 times. The rate of appearance of moxifloxacin permeates the cornea at 3.6 times the rate of gatifloxacin, and appears in the endothelial side a half less time.

In the permeability experiments, the corneas were mounted in perfusion cells as described by (Noslein (2), After a one-hour perfusion with BSS solution, the commercial preparations Vigamox™ and Zymar™ were applied to the epithelial side of the cornea for 5 min followed by rinsing, and then a 5 min exposure to carboxyfluorescein (CF). The perfusate was collected from the endothelial side of the cornea over a two-hour period, and the level of CF was determined using fluorescence spectrophotometry.

Discussion

The concentration of moxifloxacin in Vigamox™ is 0.5%, while the concentration of gatifloxacin in Zymar™ is 0.3%. Based upon the corneal penetration results in our steady state conditions, the expected advantage of moxifloxacin over gatifloxacin is 3.6 times (3.6 x 0.5/0.3). However, with the application of the commercial preparations, the advantage of moxifloxacin is 2.3 times, suggesting that the corneal integrity of the cornea during the 5 min exposure to Zymar™. Thus accompanying this 2.3 times moxifloxacin permeates the cornea at 3.6 times the rate of gatifloxacin, and appears in the endothelial side a half less time.

Conclusions

- The ex vivo corneal permeation of moxifloxacin in BSS solution is 3.6 times greater than gatifloxacin. In addition, moxifloxacin appears on the endothelial side of the cornea 2 times faster than gatifloxacin.

- The penetration of moxifloxacin is 2.3 times greater than gatifloxacin when using a 5 min dosing regimen of the fluoroquinolones from their marketed formulations Vigamox™ and Zymar™.

- However, the corneal permeability to carboxyfluorescein is 1.6 times lower for Vigamox™ compared to Zymar™. This demonstrates superior corneal penetration of moxifloxacin is due to the inherent characteristics of the moxifloxacin molecule, and is not due to changes in the corneal epithelial intracellular tight junctions.

References

1. R.D. Schoenwald and H.S. Huang, J. of Pharmaceutical Sciences, 72, 11, 1266-1271, 1983.

2. S. Klein and D.M. Maurice, J. Physiol., 221, 29-41, 1972.

Figure 1. Corneal Penetration of Moxifloxacin and Gatifloxacin Under Steady State Conditions

Table 1. Permeability Coefficients and Lag Times

Fluoroquinolone	Apparent Corneal Permeability Coefficient (x 10⁶ cm/sec)	Lag Time (min)
Moxifloxacin	91 ± 3	49 ± 1
Gatifloxacin	25 ± 2	99 ± 12

Figure 2. Corneal Penetration of Moxifloxacin and Gatifloxacin from Commercial Ophthalmic Solutions: VIGAMOX™ and ZYMAR™

Table 2. Rates of Accumulation and Lag Times

Fluoroquinolone Ophthalmic Solution	Rate of Accumulation (µg/cm/min)	Lag Time (min)
VIGAMOX™	6.5 ± 1.8	11 ± 4
ZYMAR™	2.8 ± 0.3	18 ± 5

Figure 3. Corneal Permeability to Carboxyfluorescein of Moxifloxacin and Gatifloxacin from Commercial Ophthalmic Solutions: VIGAMOX™ and ZYMAR™

Table 3. Rates and Peaks of Accumulation

Fluoroquinolone Ophthalmic Solution	Initial Rate of Accumulation (µMol/min)	Carboxyfluorescein (µMol/min)	Peak of Accumulation (µMol)
Control	0.8 ± 0.4		18 ± 8
VIGAMOX™	2.1 ± 0.6		37 ± 9
ZYMAR™	3.4 ± 0.6		60 ± 5

Aqueous Penetration and Biological Activity of Moxifloxacin 0.5% Ophthalmic Solution and Gatifloxacin 0.3% Solution in Cataract Surgery Patients

Dianne H. Kim, MD,[1] Walter J. Stark, MD,[1] Terrence P. O'Brien, MD,[1] James D. Dick, PhD[2]

Purpose: To measure the achievable perioperative aqueous concentration of the commercially available topically administered fourth generation fluoroquinolones, moxifloxacin 0.5% ophthalmic solution, and gatifloxacin 0.3% ophthalmic solution, and to correlate this concentration with the agents' biological efficacy in the aqueous humor of patients undergoing routine cataract surgery.
   Design: Prospective, randomized, parallel, double-masked, clinical trial.
   Participants: Fifty patients undergoing cataract extraction.
   Methods: Patients (n = 25) were given perioperative topical moxifloxacin 0.5% or topical gatifloxacin 0.3% (n = 25). One drop of antibiotic was administered every 10 minutes for 4 doses beginning 1 hour prior to surgery. Aqueous humor was sampled via paracentesis and antibiotic concentrations were determined using validated high performance liquid chromatography (HPLC) procedures. Dilution analyses were performed to determine the biological efficacy of the agents in the aqueous against Staphylococcus epidermidis, the most common cause of postcataract endophthalmitis.
   Main Outcome Measures: Aqueous humor antibiotic concentrations were measured using HPLC and microdilution bioassay techniques. Biological activity was measured as minimal inhibitory dilution and minimal bactericidal dilution.
   Results: Aqueous humor concentrations for moxifloxacin via HPLC analysis were 1.80 (±1.21) μg/ml, whereas those for gatifloxacin were 0.48 (±0.34) μg/ml. This 3.8-fold difference in aqueous humor antibiotic concentrations was statistically significant (P = 0.00003). Similarly, the biological dilution analysis of the aqueous humor samples showed that moxifloxacin attained an estimated activity of 2.1 μg/ml, whereas the gatifloxacin activity was approximately 0.4 μg/ml, which represented a 4.9-fold difference.
   Conclusions: This study demonstrated that after topically administered perioperative antibiotics with cataract surgery, moxifloxacin 0.5% ophthalmic solution achieved a statistically significantly higher concentration in aqueous humor compared with gatifloxacin (P = 0.00003). Results from the broth dilution analysis showed that moxifloxacin 0.5% was biologically more active against S. epidermidis than gatifloxacin 0.3% in aqueous humor after topical application. There were no adverse events reported, and incision wounds healed quickly and as expected. Ophthalmology 2005;112:1992–1996 © 2005 by the American Academy of Ophthalmology.

Recent reports indicate that endophthalmitis rates after cataract surgery are on the rise (McDonnell PJ. Endophthalmitis risk factors: clear corneal incision? Presented at: American Academy of Ophthalmology meeting, October 25, 2004; New Orleans, Louisiana). The outcome of this intraocular infection can be devastating to the patient and result in significant loss of vision and even loss of the eye. Goals of perioperative administration of antibiotics

are to prevent infection by decreasing colonization of the ocular surface normal flora and pathogens as well as to achieve a therapeutic intraocular antibiotic concentration.[1–3] Gram-positive pathogens are the most common organisms implicated in endophthalmitis cases.[4] Recently, there has been an emergence of resistant gram-positive organisms recovered from cases of endophthalmitis.[5–12]

APPENDIX I

The fourth generation fluoroquinolone eyedrops have been developed to broaden the spectrum of antibiotic coverage, including resistant strains. These molecules have a lower propensity to promote the development of resistance, because they require 2 mutations to establish resistance, one in the topoisomerase IV gene and a second one in the DNA gyrase (topoisomerase II) gene.[6,7] In addition, the bulky C-7 substituent of the moxifloxacin molecule renders it a poor substrate for bacterial efflux pumps, which effectively prevents it from being removed from the bacterial cell.[13] Therefore, more of the antibiotic accumulates within the bacterial cell resulting in rapid bacterial cell death. Gatifloxacin contains a methoxy substituent at position 8 of the quinolone ring that has been associated in some bacteria with increased bacteriostatic and bactericidal activity, as well as decreased selection of resistant mutants.[14]

In vivo penetration studies have been conducted with all of the ophthalmic fluoroquinolones: both second generation fluoroquinolones, ciprofloxacin and ofloxacin, the third generation fluoroquinolone, levofloxacin, as well as with the fourth generation fluoroquinolones, moxifloxacin and gatifloxacin (Invest Ophthalmol Vis Sci 44[suppl]:1454, 2003; Invest Ophthalmol Vis Sci 44[suppl]:2117, 2003; Invest Ophthalmol Vis Sci 45[suppl]:4906, 2004).[15,16] In studies with human patients, it has been demonstrated that moxifloxacin can safely penetrate the cornea and achieve a higher concentration in the aqueous at least 2 to 3 times that of other fluoroquinolones (Invest Ophthalmol Vis Sci 46[suppl]:5051, 2005).[17–19]

The purpose of this study was to measure the perioperative aqueous concentration of the fourth generation fluoroquinolones, moxifloxacin and gatifloxacin and to correlate this concentration with the biological activity of the agent within the aqueous specimen against the most common endophthalmitis-causing organism, *S. epidermidis*. This biological activity took into account the protein binding and other host factors that could affect the in vivo activity of the fluoroquinolone. To our knowledge, this is the first in vivo study to look at both the achievable concentration and the relative biological activity of these 2 newer generation 8-methoxy fluoroquinolone topical ocular antibiotics. Therefore, we present 2 different assessments of penetration and biological efficacy from aqueous humor samples in patients undergoing cataract surgery, both corroborating the same surgical outcome of higher potency and penetration with moxifloxacin 0.5% ophthalmic solution.

Patients and Methods

This was a prospective, randomized, parallel, double-masked, clinical trial involving 50 patients undergoing cataract extraction who were given perioperative topical moxifloxacin 0.5% (Vigamox, Alcon Laboratories, Inc., Fort Worth, TX; n = 25) or gatifloxacin 0.3% (Zymar, Allergan Inc., Irvine, CA; n = 25) commercially-available ophthalmic solutions. Institutional Review Board/Ethics Committee approval was obtained. Surgical methods were previously described in a preliminary report.[20] On the day of surgery, patients were randomly assigned to receive drops 10 minutes apart for a total of 4 doses with the last dose given (±2) minutes prior to the time of initiating the cataract incision. A 15-degree super-

blade was used to make a paracentesis, and a 30-gauge cannula on a tuberculin syringe then was used to acquire the aqueous specimen immediately through the paracentesis site. Once the specimen was acquired, it was transferred immediately to an Eppendorf tube using sterile gloves and stored in –70° C.

Moxifloxacin and gatifloxacin concentrations in human aqueous humor were determined by an independent laboratory using a validated high performance liquid chromatography (HPLC)-tandem mass spectrometry method as previously described.[18] Briefly, 50 μl of human aqueous humor was spiked with a tetra-deuterated moxifloxacin internal standard. The samples were prepared using reversed-phase, solid-phase extraction cartridges. The HPLC was performed on a reversed-phase C8 column.

Broth Dilution Assay

The bactericidal activity of the aqueous humor was determined according to the National Committee for Clinical Laboratory Standards guideline for performance of the serum bactericidal test.[21] This methodology is useful in assessing the inhibitory and lethal activity of antibiotics in vivo, because it takes into consideration host factors such as penetration and protein binding as well as organism–antibiotic interaction. For analysis, 0.1 ml of each aqueous humor sample was serially diluted 2-fold in 0.1 ml of cat ion adjusted Mueller–Hinton broth in sterile microtiter plates over a dilution range of 1:2 through 1:128. The reference organism used to determine the bactericidal activity of the aqueous samples was a clinical isolate of *S. epidermidis*, the most common causative organism for postcataract endophthalmitis. The reference *S. epidermidis* utilized in these experiments exhibited a minimal inhibitory concentration (MIC) of 0.1 μg/ml to gatifloxacin and an MIC of 0.05 μg/ml to moxifloxacin. These MIC values are consistent with those found in the published literature.[6] For analysis, the reference strain was grown overnight on trypticate soy agar with 5% sheep blood, 3 to 5 colonies were inoculated into CAMHB, and incubated for 6 hours at 35° C, and then the inoculum broth culture was adjusted to 0.5 MacFarland standard (~1–5×10^{8} CFU/ml) diluted 1:10 in CAMHB and 0.01 ml and was added to each microtiter well to yield a final concentration of 1–5×10^{4}) CFU/microtiter well. Trays were incubated in ambient air at 35° C for 24 hours. Each specimen was read for turbidity and the highest dilution demonstrating no growth was determined as the minimal inhibitory dilution. Wells showing no turbidity were quantitatively (0.1 ml) subcultured to trypticase soy agar with 5% sheep blood and incubated for 24 hours at 35° C. After incubation, the colonies were counted, and the minimal bactericidal dilution was determined as the highest dilution yielding ≤ 10 colonies (>99.9% Killing). For purposes of estimating the antibiotic concentration in each sample, the minimal inhibitory dilution was multiplied by the MIC of 0.05 μg/ml for moxifloxacin and 0.1 μg/ml for gatifloxacin of the reference *S. epidermidis*.

Statistical analyses were performed using a Student's *t* test to detect differences between the antibiotic treatment groups.

Results

Mean aqueous humor measured concentrations obtained via HPLC analysis for moxifloxacin were 1.80 (±1.21) μg/ml compared with for 0.48 (±0.34) μg/ml gatifloxacin (Fig 1). This represented a 3.8-fold difference in measured aqueous humor antibiotic concentrations, which was statistically significant (*P* = 0.00003).

Microbiological dilution bioassay of the aqueous humor samples showed that moxifloxacin attained an estimated antibiotic concentration based on inhibitory activity of 2.1 μg/ml (±1.7 μg/ml), whereas the gatifloxacin concentration was 0.4 μg/ml (±0.4
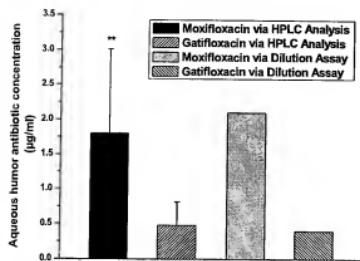
Figure 1. Aqueous humor antibiotic concentrations and dilution bioassay results from patients undergoing cataract surgery. One drop of antibiotic was administered every 10 minutes for 4 doses beginning 1 hour prior to surgery. The aqueous humor sample was collected at the time of incision within a 30 (±2) minute window from the time of the last antibiotic drop. Moxifloxacin achieved an aqueous humor concentration of 1.80 μg/ml, whereas gatifloxacin was 0.48 μg/ml. This difference was significantly different (**P = 0.00003). The aqueous humor concentrations were estimated as 2.1 μg/ml for moxifloxacin and 0.4 μg/ml for gatifloxacin by multiplying the minimal inhibitory dilution by the minimal inhibitory concentration of the reference Staphylococcus epidermidis. HPLC = high performance liquid chromatography.

μg/ml, Fig 1). Dilutional analysis resulted in an average minimal inhibitory dilution of 1:42 for moxifloxacin 0.5% and 1:4 for gatifloxacin 0.3% against S. epidermidis. The average minimal bactericidal dilution was 1:40 for moxifloxacin 0.5% ophthalmic solution and 1:3 for gatifloxacin 0.3% ophthalmic solution. Thus, moxifloxacin aqueous samples had to be diluted to a higher extent than gatifloxacin aqueous samples before bacterial growth was observed. These results showed that moxifloxacin 0.5% was biologically more active against S. epidermidis than gatifloxacin 0.3% in aqueous humor after topical application. There were no adverse events reported, and incision wounds healed quickly and as expected.

Discussion

Recent reports indicate that resistance to earlier generation ocular antibiotics among clinical bacterial isolates is becoming more prevalent.[2,7–12,22,23] The increasing number of ocular surgical procedures poses a greater risk for perioperative infection. Recent reports indicate an upward trend in the incidence of bacterial infections after cataract and refractive surgery.[24–26] This increased risk of surgical complications, such as postoperative endophthalmitis and keratitis, in part prompted the advance of the fourth-generation antibiotics moxifloxacin 0.5% ophthalmic solution and gatifloxacin 0.3% ophthalmic solution for the prevention and treatment of these potentially serious ocular infections. Moxifloxacin and gatifloxacin are 8-methoxy-substituted fluoroquinolone agents demonstrating greater potency against gram-positive organisms, and including for the first time, certain species of atypical mycobacteria compared with earlier generation fluoroquinolones while retaining ex-

cellent coverage against gram-negative bacteria.[6,7] Specific to endophthalmitis, Kowalski et al[27] demonstrated a first proof-of-principle for prophylactic topical fluoroquinolone antibiotic use in the prevention of endophthalmitis in an animal model demonstrating that pretreatment with moxifloxacin prevented development of endophthalmitis after a large intravitreal inoculum of bacteria administered to rabbits.

An important factor that contributes to the success of antibiotic therapy is the ability of the molecule to penetrate the target ocular tissues at concentrations greater than the MIC. The mutant prevention concentration is typically 8- to 10-fold higher than the MIC for a given organism.[28,29] Recent studies indicate the likelihood of selection for resistant microorganisms can be reduced by maintaining concentrations at or greater than the mutant prevention concentration.[30] Therefore, maintaining the highest possible ratio between aqueous humor fluoroquinolone concentrations and MIC, preferably at or greater than the mutant prevention concentration decreases the probability of selecting for single-step mutants.[7,31] Moxifloxacin 0.5% ophthalmic solution provided drug penetration at concentrations greater than the MICs for S. epidermidis, Streptococcus pneumoniae, viridans streptococci, enterococci, and Bacillus species, as well as fluoroquinolone-susceptible and resistant Staphylococcus aureus (Fig 2).[6] Gatifloxacin achieved MICs for all of these organisms, except for fluoroquinolone-resistant S. aureus (Fig 3).

The current penetration study corroborates reports from other investigators with animal models and human patients. Moxifloxacin (distribution coefficient at pH 7.4 = 0.61) is more lipophilic than gatifloxacin (distribution coefficient at pH 7.4 = 0.11) (Invest Ophthalmol Vis Sci 45[suppl]:4907, 2004). This facilitates the ability of moxifloxacin to traverse both the epithelial and endothelial corneal membrane layers. Tissue penetration studies with excised rabbit corneas demonstrated that moxifloxacin 0.5% safely produced a 3.6-fold
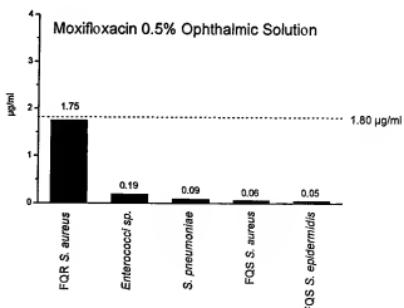
Figure 2. Moxifloxacin gram-positive minimal inhibitory concentrations in relation to aqueous humor concentrations from the current study. Minimal inhibitory concentration values are from Mather et al.[6] FQR = fluoroquinolone resistant; FQS = fluoroquinolone sensitive.
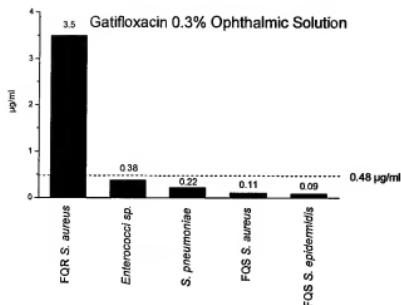
Figure 3. Gatifloxacin gram-positive minimal inhibitory concentrations in relation to aqueous humor concentrations from the current study. Minimal inhibitory concentration values are from Mather et al.[6] FQR = fluoroquinolone resistant; FQS = fluoroquinolone sensitive.

higher corneal penetration coefficient than gatifloxacin 0.3% (Invest Ophthalmol Vis Sci 45[suppl]:4910, 2004). The appearance of moxifloxacin on the endothelial side was also approximately 2-fold faster than gatifloxacin. Another rabbit study demonstrated that moxifloxacin was readily absorbed into the aqueous humor and anterior ocular tissues (Invest Ophthalmol Vis Sci 44[suppl]:1454, 2003). Solomon et al[17] conducted a prospective study in cataract patients in which the antibiotic dosing frequency was 4 times daily for 3 days prior to surgery. On the day of surgery, patients received the antibiotics every 15 minutes for 3 doses 1 hour prior to their procedure. Aqueous humor concentrations at the time of surgery were significantly higher for moxifloxacin (P<0.05) than for gatifloxacin. Katz et al[18] measured moxifloxacin aqueous humor concentrations in cataract patients who received 1 drop every 15 minutes for 4 doses before surgery (group 1), or 1 drop 4 times a day the day before surgery plus the same preoperative regimen as group 1 (group 2). The maximal concentration achieved with these 2 regimens was not significantly different. The area under the aqueous concentration-time curve ($AUC_{0-3\,hr}$) did show a difference in favor of group 2 (P = 0.04). This study also corroborates that topical moxifloxacin was well absorbed into the aqueous humor at concentrations much greater than the median MICs for common pathogens involved in endophthalmitis. In a prospective study of cataract patients dosed 4 times a day the day before surgery with 1 additional drop an hour before surgery, McCulley's group reported aqueous humor concentrations that were significantly higher for moxifloxacin than for gatifloxacin (Invest Ophthalmol Vis Sci 46[suppl]: 5051, 2005). The antibiotic concentrations attainable with topical dosing reported from these published studies are consistent with those from the current study that were measured via HPLC as well as through a microdilution bioassay.

To the best of our knowledge, there are no published studies that have compared the efficacy of achievable concentrations of moxifloxacin 0.5% ophthalmic solution with gatifloxacin 0.3% ophthalmic solution in aqueous humor via broth dilution.

Clinical studies with human patients confirm the preclinical studies with moxifloxacin and gatifloxacin that demonstrate that effective corneal penetration does not compromise the safety of these antibiotics (Invest Ophthalmol Vis Sci 46[suppl]:4903, 2005).[32–34] Yee et al's[35] group recently reported that there were no significant differences in human corneal wound healing, haze, or visual acuity between moxifloxacin 0.5% and gatifloxacin 0.3% (dosed every 6 hours until complete wound healing had occurred) for bilateral photorefractive keratectomy patients. Another study from the same laboratory showed equivalence between these 2 fluoroquinolone antibiotics (dosed 4 times a day for 3 days prior to surgery and 7 days postoperatively) in terms of flap clarity, stromal edema, flap edema, epithelial defect, and visual acuity for Epi-LASIK patients (Invest Ophthalmol Vis Sci 46[suppl]:4877, 2005). Durrie and Trattler[36] also reported that moxifloxacin 0.5% and gatifloxacin 0.3% ophthalmic solutions were equivalent in terms of corneal healing after LASIK and laser epithelial keratomileusis surgery. Thus, both products are believed to be biocompatible when administered in doses recommended by prevailing standards-of-care in a variety of ophthalmic surgical procedures.

The current study presents 2 assessments of fluoroquinolone penetration and biological efficacy from aqueous humor samples in patients undergoing cataract surgery. These results corroborate the same statistical and clinical outcomes of higher potency and therapeutic penetration for moxifloxacin 0.5% ophthalmic solution compared with gatifloxacin 0.3% ophthalmic solution. In our study, both moxifloxacin 0.5% and gatifloxacin 0.3% exceeded the known MIC values for most pathogens that cause endophthalmitis. The higher aqueous levels of moxifloxacin 0.5% may provide greater efficacy especially against fluoroquinolone resistant S. aureus.

References

1. Ciulla TA, Starr MB, Masket S. Bacterial endophthalmitis prophylaxis for cataract surgery: an evidence-based update. Ophthalmology 2002;109:13–24.
2. Mino de Kaspar H, Chang RT, Shriver EM, et al. Three-day application of topical ofloxacin reduces the contamination rate of microsurgical knives in cataract surgery: a prospective randomized study. Ophthalmology 2004;111:1352–5.
3. Snyder-Perlmutter LS, Katz HR, Melia M. Effect of topical ciprofloxacin 0.3% and ofloxacin 0.3% on the reduction of bacterial flora on the human conjunctiva. J Cataract Refract Surg 2000;26:1620–5.
4. Han DP, Wisniewski SR, Wilson LA, et al. Spectrum and susceptibilities of microbiologic isolates in the Endophthalmitis Vitrectomy Study. Am J Ophthalmol 1996;122:1–17.
5. Kowalski RP, Dhaliwal DK, Karenchak LM, et al. Gatifloxacin and moxifloxacin: an in vitro susceptibility comparison to levofloxacin, ciprofloxacin, and ofloxacin using bacterial keratitis isolates. Am J Ophthalmol 2003;136:500–5.

6. Mather R, Karenchak LM, Romanowski EG, Kowalski RP. Fourth generation fluoroquinolones: new weapons in the arsenal of ophthalmic antibiotics. Am J Ophthalmol 2002;133: 463–6.

7. Hwang DG. Fluoroquinolone resistance in ophthalmology and the potential role for newer ophthalmic fluoroquinolones. Surv Ophthalmol 2004;49(suppl):S79–83.

8. Alexandrakis G, Alfonso EC, Miller D. Shifting trends in bacterial keratitis in south Florida and emerging resistance to fluoroquinolones. Ophthalmology 2000;107:1497–502.

9. Goldstein MH, Kowalski RP, Gordon YJ. Emerging fluoroquinolone resistance in bacterial keratitis: a 5-year review. Ophthalmology 1999;106:1313–8.

10. Mah FS. New antibiotics for bacterial infections. Ophthalmol Clin North Am 2003;16:11–27.

11. Mah FS. Fourth-generation fluoroquinolones: new topical agents in the war on ocular bacterial infections. Curr Opin Ophthalmol 2004;15:316–20.

12. Marangon FB, Miller D, Muallem MS, et al. Ciprofloxacin and levofloxacin resistance among methicillin-sensitive Staphylococcus aureus isolates from keratitis and conjunctivitis. Am J Ophthalmol 2004;137:453–8.

13. Pestova E, Millichap JJ, Noskin GA, Peterson LR. Intracellular targets of moxifloxacin: a comparison with other fluoroquinolones. J Antimicrob Chemother 2000;45:583–90.

14. Fukuda H, Kishii R, Takei M, Hosaka M. Contributions of the 8-methoxy group of gatifloxacin to resistance selectivity, target preference, and antibacterial activity against Streptococcus pneumoniae. Antimicrob Agents Chemother 2001;45:1649–53.

15. Yalvac IS, Basci NE, Bozkurt A, Duman S. Penetration of topically applied ciprofloxacin and ofloxacin into the aqueous humor and vitreous. J Cataract Refract Surg 2003;29: 487–91.

16. Bezwada P, Clark L, Adams S, et al. Comparative ocular bioavailability and efficacy of topical levofloxacin and ofloxacin in rabbits. J Toxicol Cutaneous Ocul Toxicol 2004;23:83–90.

17. Solomon R, Donnenfeld ED, Perry HD, et al. Penetration of topically applied gatifloxacin 0.3%, moxifloxacin 0.5%, and ciprofloxacin 0.3% into the aqueous humor. Ophthalmology 2005;112:466–9.

18. Katz HR, Masket S, Lane SS, et al. Absorption of topical moxifloxacin ophthalmic solution into human aqueous humor. Cornea. In press.

19. Donnenfeld ED, Schrier A, Perry HD, et al. Penetration of topically applied ciprofloxacin, norfloxacin, and ofloxacin into the aqueous humor. Ophthalmology 1994;101:902–5.

20. Kim DH, Stark WJ, O'Brien TP. Ocular penetration of moxifloxacin 0.5% and gatifloxacin 0.3% ophthalmic solutions into the aqueous humor following topical administration prior to routine cataract surgery. Curr Med Res Opin 2005; 21:93–4.

21. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 3rd ed. Villanova, PA: National Committee for Clinical Laboratory Standards; 1993. Approved standard M7-A3.

22. Chaudhry, NA, Flynn HW Jr, Murray TG, et al. Emerging ciprofloxacin-resistant Pseudomonas aeruginosa. Am J Ophthalmol 1999;128:509–10.

23. Smith RD, Coast J. Antimicrobial resistance: a global response. Bull World Health Organ 2002;80:126–33.

24. Colleaux KM, Hamilton WK. Effect of prophylactic antibiotics and incision type on the incidence of endophthalmitis after cataract surgery. Can J Ophthalmol 2000;35:373–8.

25. Karp CL, Tuli SS, Yoo SH, et al. Infectious keratitis after LASIK. Ophthalmology 2003;110:503–10.

26. Nagaki Y, Hayasaka S, Kadoi C, et al. Bacterial endophthalmitis after small-incision cataract surgery: effect of incision placement and intraocular lens type. J Cataract Refract Surg 2003;29:20–6.

27. Kowalski R, Romanowski EG, Mah FS, et al. Topical prophylaxis with moxifloxacin prevents endophthalmitis in a rabbit model. Am J Ophthalmol 2004;138:33–7.

28. Schentag JJ. Pharmacokinetic and pharmacodynamic predictors of antimicrobial efficacy: Moxifloxacin and Streptococcus pneumoniae. J Chemother 2002;14(Suppl):13–21.

29. Metzler K, Hansen GM, Hedlin P, et al. Comparison of minimal inhibitory and mutant prevention drug concentrations of 4 fluoroquinolones against clinical isolates of methicillin-susceptible and -resistant Staphylococcus aureus. Int J Antimicrob Agents 2004;24:161–7.

30. Blondeau JM, Zhao X, Hansen G, Drlica K. Mutant prevention concentrations of fluoroquinolones for clinical isolates of Streptococcus pneumoniae. Antimicrob Agents Chemother 2001;45:433–8.

31. Zhao X, Drlica K. Restricting the selection of antibiotic-resistant mutants: a general strategy derived from fluoroquinolones studies. Clin Infect Dis 2001;33(Suppl):S147–56.

32. Kovoor TA, Kim AS, McCulley JP et al. Evaluation of the corneal effects of topical ophthalmic fluoroquinolones using in vivo confocal microscopy. Eye Contact Lens 2004;30:90–4.

33. Herrygers LA, Noecker RJ, Lane LC, Levine JM. Comparison of corneal surface effects of gatifloxacin and moxifloxacin using intensive and prolonged dosing protocols. Cornea 2005; 24:66–71.

34. Burka JM, Bower KS, VanRoekel RC et al. The effect of 4th generation fluoroquinolones gatifloxacin and moxifloxacin on epithelial healing following photorefractive keratectomy. Am J Ophthalmol 2005;140:83–7.

35. Yee RW, Pete Setabutr P, Foltermann MO, et al. The effects of topical moxifloxacin 0.5% ophthalmic solution and gatifloxacin 0.3% solution on corneal healing following bilateral photorefractive keratectomy (PRK). Cornea. In press.

36. Durrie D, Trattler W. A comparison of therapeutic regimens containing moxifloxacin 0.5% ophthalmic solution and gatifloxacin 0.3% ophthalmic solution for surgical prophylaxis in patients undergoing LASIK or LASEK. J Ocular Pharm Ther 2005;21:236–41.

In re: Cagle, et al.

Serial No. 10/715,055

Confirmation No. 3314

Filed: November 17, 2003

Group Art Unit: 1618

Examiner: Z. Fay

For: "Method of Treating Ophthalmic Infections with Moxifloxacin Compositions"

DECLARATION UNDER 37 CFR §1.132

Customer Service Window
Randolph Building
401 Dulany Street
Alexandria, VA 22314

Dear Sir:

I, Geoffrey R. Owen, hereby say and declare as follows.

1. I received my bachelor's degree in the Natural Science Tripos from Cambridge University, England, in 1968. I received my doctorate degree in organic chemistry from Cambridge University in 1971. Since 1994, I have worked at Alcon Laboratories, Inc. or Alcon Research, Ltd. (collectively "Alcon"), where I have held various positions in the Optical Research, Consumer Products Research, and Pharmaceutical Research Groups.

2. My title at Alcon is Technical Director, Pharmaceutical Research, a position I have held since 2000. My responsibilities include designing, preparing, and testing topical ophthalmic pharmaceutical formulations. Pharmacokinetic studies of topical ophthalmic products and product candidates are a routine part of my job responsibilities in the Pharmaceutical Research Group, and I have extensive

experience designing, preparing, conducting and analyzing studies of fluoroquinolone antibiotic compounds. Since 2000, I have published 11 articles relating to ocular pharmacokinetics of topical ophthalmic drug products, 8 of which relate to fluoroquinolones. My experience with ophthalmic formulations in general has been continuous since 1990.

3.   I am aware that U.S. Patent Application No. 10/715,055 (the Application) is directed, in part, to methods of treating ophthalmic infections via topical administration of compositions containing moxifloxacin at a concentration of 0.1 to 1.0 wt. %.

4.   I have reviewed the Declaration of Dr. David Stroman dated May 22, 2007 ("Dr. Stroman's Declaration), and understand that it has been considered by the U.S. Patent and Trademark Office ("USPTO") in connection with the examination of the Application. I also understand that the Examiner reviewing the Application on behalf of the USPTO has requested that the Applicants provide additional data to support their contention that moxifloxacin compositions exhibit superior ocular penetration properties over the entire concentration range recited in the Application's claims (i.e., 0.1 to 1.0 wt. %).

5.   Ocular penetration studies are routinely conducted using an *ex vivo* corneal penetration model that was described by Schoenwald and Huang in an article published in the <u>Journal of Pharmaceutical Sciences</u> in 1983. This article is cited in Dr. Stroman's Declaration in Paragraph 18, and is attached to Dr. Stroman's Declaration as Appendix F. This corneal penetration model ("the Steady-State Model"), which uses a corneal perfusion chamber and rabbit cornea, is well accepted as an accurate and reliable representation of ocular penetration *in vivo*. The Schoenwald, et al. article has been cited more than 200 times in the scientific literature.

6.   All of the testing presented in this paragraph and the remainder of this Declaration was performed by me or under my direction. Formulations of various fluoroquinolone compounds were prepared by adding to a buffered saline solution having a physiological pH (pH = 7.3) an amount of fluoroquinolone equivalent to 0.1 mmol (~ 0.004 wt. %)[1]. These formulations were then tested using the Steady-State Model to investigate their corneal penetration properties. The results are shown in Table 1 and Figure 1 below.

TABLE 1

Ex-Vivo Corneal Perfusion Chambers
Fluoroquinolone Comparison
0.1 mmol (~0.004%) Solutions at pH 7.3

Fluoroquinolone	No. of eyes	Rate ($\times10^{-2}$ µg/min)	300 minute Accumulation (µg)	Lag Time (min)	Permeability Coefficient * ($\times10^{-7}$ cm/sec)
Moxifloxacin	3	2.9 ± 0.3	7.0 ± 0.5	59 ± 6	111 ± 10
Ofloxacin	4	1.2 ± 0.1	2.8 ± 0.3	74 ± 2	53 ± 5
Lomefloxacin	3	1.0 ± 0.02	2.2 ± 0.1	91 ± 3	45 ± 1
Levofloxacin	3	0.70 ± 0.09	1.6 ± 0.2	76 ± 10	30 ± 4
Gatifloxacin	3	0.65 ± 0.04	1.2 ± 0.1	115 ± 4	27 ± 2
Ciprofloxacin	5	0.55 ± 0.07	1.1 ± 0.1	84 ± 12	25 ± 3
Norfloxacin	3	0.48 ± 0.02	1.0 ± 0.1	82 ± 7	23 ± 1

* These values were calculated using the original concentrations
of the fluoroquinolones in the donor (epithelial) chamber.

[1] All of the testing presented in this Declaration compared formulations containing equivalent concentrations of the respective fluoroquinolones.

FIGURE 1

**Ex-Vivo Corneal Perfusion Chambers
Rate of Diffusion (Flux) of Fluoroquinolones
0.1 mmol (~ 0.004%) Solutions at pH 7.3**
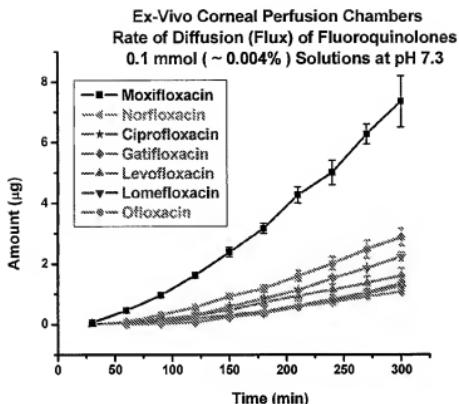
7.   The data in Table 1 and Figure 1 show a rank order of ocular penetration, with moxifloxacin solution being superior to all of the others.   These results, which were generated in approximately 2003, have been published, in part (i.e., the data relating to moxifloxacin and gatifloxacin), in the following two publications: the series of articles published as a Special Supplement to the November 2005 edition of <u>Survey of Ophthalmology</u>, International Review Journal (volume 50, supplement 1) and the abstract published as "Corneal penetration and changes in corneal permeability of moxifloxacin versus gatifloxacin" in <u>Invest. Ophthalmol. Vis. Sci.</u> 2004 45: E-Abstract 4910.   A copy of the <u>Survey of Ophthalmology</u> publication was attached to Dr. Stroman's Declaration as Appendix E. A copy of the <u>Invest. Ophthalmol. Vis. Sci.</u> publication is attached to the present Declaration as Appendix A.

8.   The Steady-State Model is predictive of the relative ocular penetration properties of drug compositions tested at identical concentrations, and this is true

4

regardless of the drug concentration used in the test. Nevertheless, I understand that the Examiner reviewing the Application has requested comparisons of moxifloxacin compositions to other fluoroquinolone compositions at drug concentrations greater than 0.5 wt.%. In response to that request, I performed additional tests at drug concentrations of 0.1, 0.3, 0.5, 0.75 and 1.0 wt. %, as described below.

9.   Ideally, topical ophthalmic formulations have a pH as close to physiological pH (~pH 7.3) as possible. However, some drugs are not sufficiently soluble at that pH to permit comparative testing at identical drug concentrations. In those cases, it is necessary to reduce the pH for the insufficiently soluble fluoroquinolone to a point where the desired drug concentration can be achieved. The Steady-State Model was used to generate comparative corneal penetration data for the indicated fluoroquinolones at drug concentrations of 0.1, 0.3. 0.5, 0.75 and 1.0 wt.%, and the results are shown in Tables 2A, 2B, 2C, 2D, and 2E, respectively.[2] The results are also presented in graphical form in Figures 2A, 2B, 2C, 2D, and 2E, respectively. These results clearly demonstrate the significantly superior corneal penetration properties exhibited by moxifloxacin compositions over compositions containing the other tested fluoroquinolones.

[2] All fluoroquinolones were tested in buffered saline solution (pH = 7.3) unless the desired drug concentration could not be obtained. In some cases, a target concentration of a given fluoroquinolone could not be reached without damaging the cornea used in the Steady-State Model because the solution pH would have had to be adjusted to a pH that was too acidic to permit the rabbit cornea to survive. Even though it involved a less than ideal pH, a comparison of compositions containing moxifloxacin to ofloxacin and levofloxacin was also performed at a fixed pH of 5.8 and at drug concentrations of 0.3, 0.5 and 0.75 wt.%. Moxifloxacin was not sufficiently soluble at pH 5.8 to obtain a 1.0 wt.% composition so no comparative test at 1.0 wt.% was conducted at pH 5.8. The results are shown in Tables B1 – B3, attached in Appendix B.

TABLE 2A

Ex-Vivo Corneal Perfusion Chambers
Fluoroquinolone Comparison
0.1% Solutions

Fluoroquinolone	pH	Rate (µg/min)	240 minute Accumulation (µg)	Lag Time (min)	Permeability Coefficient ($\times 10^{-7}$ cm/sec)
Moxifloxacin	7.3	0.74	158	25	112.9
		0.72	147	37	110.9
Ofloxacin	7.1	0.47	83.2	43	72.4
		0.4	79.8	41	61.6
Levofloxacin	7.3	0.42	87	33	64.3
		0.39	81	31	59.4
Lomefloxacin	7.1	0.46	88.3	48	70.7
		0.38	70.7	52	57.7
Gatifloxacin*	7.9	0.31	59.8	48	47.8
		0.37	72.3	46	57.2
Ciprofloxacin	4.4	0.28	67.8	48	43.4
		0.32	64.4	36	48.4

* Gatifloxacin was added to the buffered saline solution (pH 7.3) as a free base and caused the pH to increase.

Figure 2A

Ex-Vivo Corneal Perfusion Chambers
Rate of Diffusion (Flux) of Fluoroquinolones
0.1% Solutions
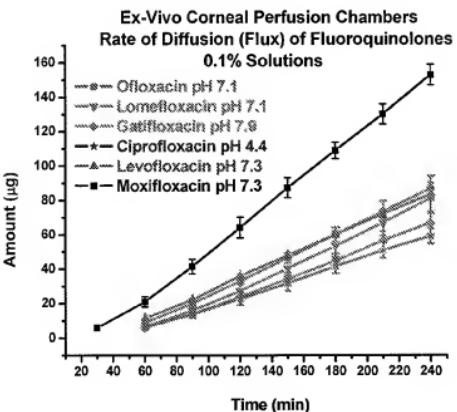
TABLE 2B

Ex-Vivo Corneal Perfusion Chambers
Fluoroquinolone Comparison
0.3% Solutions

Fluoroquinolone	pH	Rate (µg/min)	240 minute Accumulation (µg)	Lag Time (min)	Permeability Coefficient (x10⁻⁷ cm/sec)
Moxifloxacin	7.3	2.5	524	27	125.7
		2.5	540	28	130.1
Ofloxacin	6.8	1.1	231	39	68.6
		1.5	287	52	77.8
Lomefloxacin	6.7	1.4	261	48	69.6
		1.1	213	48	56.8
Levofloxacin	7.3	1.1	226	39	57.5
		1.1	215	43	55.6
Gatifloxacin	8.1	0.81	152	53	41.4
		1	202	42	52
Ciprofloxacin	4.6	0.30	59.3	45	15.6
		0.42	84.9	38	21.5

FIGURE 2B

Ex-Vivo Corneal Perfusion Chambers
Rate of Diffusion (Flux) of Fluoroquinolones
0.3% Solutions
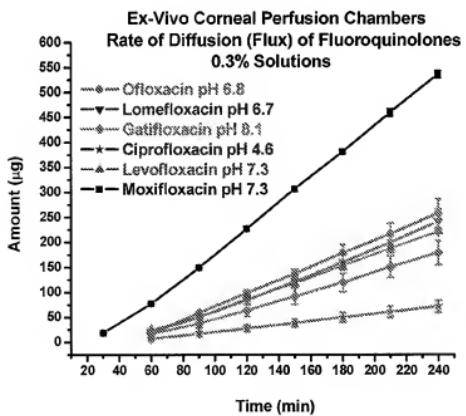
TABLE 2C

Ex-Vivo Corneal Perfusion Chambers
Fluoroquinolone Comparison
0.5% Solutions

Fluoroquinolone	pH	Rate (µg/min)	240 minute Accumulation (µg)	Lag Time (min)	Permeability Coefficient ($\times 10^{-7}$ cm/sec)
Moxifloxacin	7.3	3.7	758	38	114.7
		3.5	666	49	106.6
Ofloxacin	6.3	2.6	643	35	81.1
		3	593	40	90.8
Lomefloxacin	6.4	1.97	380	47	60.3
		1.95	369	51	59.7
Levofloxacin	7.3	1.7	344	38	52.2
		1.6	292	56	48.7
Gatifloxacin	8.1	1.8	347	47	55
		1.3	247	51	40.1
Ciprofloxacin	4.4	0.98	193	43	30.1
		0.76	146	47	23.2

FIGURE 2C

Ex-Vivo Corneal Perfusion Chambers
Rate of Diffusion (Flux) of Fluoroquinolones
0.5% Solutions

8
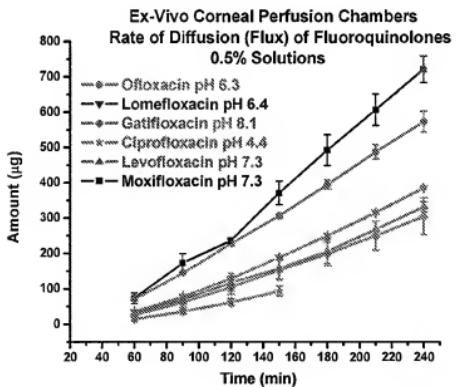
TABLE 2D

Ex-Vivo Corneal Perfusion Chambers
Fluoroquinolone Comparison
0.75% Solutions

Fluoroquinolone	pH	Rate (µg/min)	240 minute Accumulation (µg)	Lag Time (min)	Permeability Coefficient (×10⁻⁷ cm/sec)
Moxifloxacin	7.3	4.8	973	36	97.6
		4.9	981	39	99.5
Gatifloxacin*	8	2.3	432	53	47.2
		4.4	868	44	90.5
	7.3	3.3	650	43	67.5
		3.5	714	35	71.3
Levofloxacin	7.3	3.3	683	33	68
		2.5	508	39	81.7
Lomefloxacin	6.2	3.1	616	39	62.5
		2.6	506	45	52.9
Ofloxacin	6	2.5	479	51	51.9
		2.3	549	47	56
	5.9	2.5	564	36	56.3
		3.4	620	38	62.7
Ciprofloxacin	4.1	1.3	255	43	26.5
		1.1	216	44	22.5

*As in the other tests, gatifloxacin was added to the buffered saline solution (pH 7.3) as a free base and caused the pH to increase. In a second sample of the gatifloxacin solution, the pH was adjusted back to pH 7.3.

FIGURE 2D

Ex-Vivo Corneal Perfusion Chambers
Rate of Diffusion (Flux) of Fluoroquinolones
0.75% Solutions

Legend:
- Ofloxacin pH 6.0
- Lomefloxacin pH 6.2
- Ciprofloxacin pH4.1
- Levofloxacin pH 7.3
- Moxifloxacin pH 7.3
- Gatifloxacin pH 8.0 & 7.3

X-axis: Time (min)
Y-axis: Amount (µg)
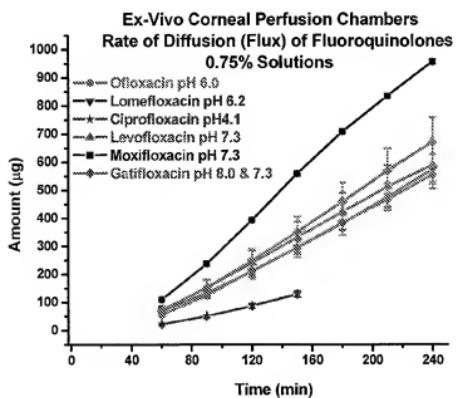
TABLE 2E

Ex-Vivo Corneal Perfusion Chambers
Fluoroquinolone Comparison
1.0% Solutions

Fluoroquinolone	pH	Rate (µg/min)	240 minute Accumulation (µg)	Lag Time (min)	Permeability Coefficient (×10⁻⁷ cm/sec)
Moxifloxacin	7.3	5.5	1191	24	84.3
		6.1	1314	26	94.1
Ofloxacin	5.8	4.1	858	33	63.5
		4.4	889	35	67.4
Levofloxacin	7.3	4	748	52	61
		4.4	875	40	67.1
Gatifloxacin	7.2	4.2	809	48	64.6
		4.1	763	54	62.9

FIGURE 2E

Ex-Vivo Corneal Perfusion Chambers
Rate of Diffusion (Flux) of Fluoroquinolones
1.0% Solutions

10
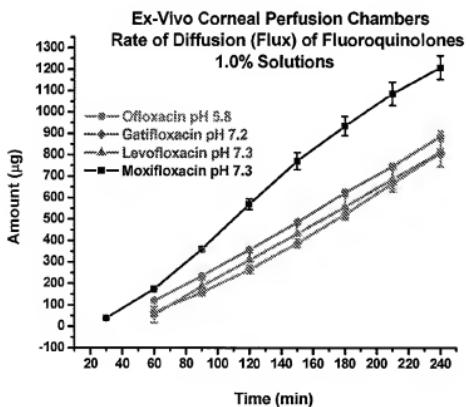
10.   The data in Tables 2A – 2E and Figures 2A – 2E show the significantly superior corneal penetration property of moxifloxacin compositions compared to the other tested fluoroquinolones. The superior results for moxifloxacin compositions are consistent across all of the tested drug concentrations tested (0.1, 0.3. 0.5, 0.75 and 1.0 wt.%).

11.   I also compared the ocular penetration properties of moxifloxacin and ofloxacin compositions in the *in vivo* rabbit model described in "A novel *in vivo* model that mimics human dosing to determine the distribution of antibiotics in ocular tissues," <u>Journal of Ocular Pharmacology and Therapeutics</u>, 23(4), 335-342 (2007). The tested compositions contained 0.5, 0.75 and 1.0 wt.% of each fluoroquinolone.[3] The results are shown in Tables 3A – 3C.

TABLE 3A
0.5 % Drug Concentration

Ocular Distribution of Fluoroquinolones
1 Hour after a Single Topical Dose

Tissue	Moxifloxacin 0.5% @ pH 7.3	Ofloxacin 0.5% @ pH 6.3	Ratio Moxi / Ofox
	Concentration (µg/g or µg/mL) Mean ± SE (n = 4)		
Aqueous Humor	2.6 ± 0.5	1.2 ± 0.2	2.1
Iris-Ciliary Body	1.7 ± 0.5	1.0 ± 0.2	1.7
Cornea	12.5 ± 2.6	7.6 ± 0.8	1.6
Upper Palpebral Conjunctiva	1.5 ± 0.5	0.5 ± 0.1	2.7
Lower Palpebral Conjunctiva	2.0 ± 1.0	0.7 ± 0.1	2.8
Bulbar Conjunctiva	1.6 ± 0.6	1.2 ± 0.2	1.3
Sclera	1.5 ± 0.3	1.2 ± 0.3	1.2

---

[3] *Because ofloxacin was not sufficiently soluble at pH 7.3, the pH of the ofloxacin composition was adjusted to permit the target drug concentration to be soluble. I also evaluated the same two fluoroquinolones at 0.5 and 0.75 wt.% in solutions having a pH of 5.8 even though the target pH for topical ocular products is physiological pH. Moxifloxacin was not sufficiently soluble at pH 5.8 to obtain a 1.0 wt.% solution. The results are shown in Table B4, attached in Appendix B. Even at this less than ideal pH (pH 5.8), superior aqueous humor concentrations of moxifloxacin were achieved. Of the sites or tissues examined in this experiment, aqueous humor drug levels provide the best indication of a drug's ability to penetrate the cornea.*

TABLE 3B
0.75 % Drug Concentration

Ocular Distribution of Fluoroquinolones
1 Hour after a Single Topical Dose

Tissue	Moxifloxacin 0.75% @ pH 7.3	Ofloxacin 0.75% @ pH 6.0	Ratio Moxi / Oflox
	Concentration (µg/g or µg/mL) Mean ± SE (n = 4)		
Aqueous Humor	2.5 ± 0.2	1.1 ± 0.2	2.3
Iris-Ciliary Body	1.3 ± 0.3	0.7 ± 0.1	2.0
Cornea	10.3 ± 1.9	6.3 ± 1.0	1.6
Upper Palpebral Conjunctiva	0.5 ± 0.2	0.4 ± 0.1	1.4
Lower Palpebral Conjunctiva	1.8 ± 0.3	0.7 ± 0.2	2.7
Bulbar Conjunctiva	4.9 ± 2.7	0.9 ± 0.2	5.2
Sclera	1.0 ± 0.7	1.0 ± 0.4	1.0

TABLE 3C
1.0 % Drug Concentration

Ocular Distribution of Fluoroquinolones
1 Hour after a Single Topical Dose

Tissue	Moxifloxacin 1.0% @ pH 7.3 (n = 5)	Ofloxacin 1.0% @ pH 5.8 (n = 3)	Ratio Moxi / Oflox
	Concentration (µg/g or µg/mL) Mean ± SE		
Aqueous Humor	3.7 ± 0.6	1.1 ± 0.3	3.2
Iris-Ciliary Body	2.0 ± 0.2	0.38 ± 0.2	3.3
Cornea	16.8 ± 2.1	6.4 ± 1.8	2.6
Upper Palpebral Conjunctiva	2.0 ± 0.3	0.6 ± 0.1	3.5
Lower Palpebral Conjunctiva	2.7 ± 0.4	0.6 ± 0.2	4.7
Bulbar Conjunctiva	2.4 ± 0.5	1.2 ± 0.2	2.0
Sclera	3.3 ± 0.6	1.6 ± 0.4	2.1

12

Figures 3A – 3C depict in bar graph form the results across all three tested drug concentrations for the aqueous humor, cornea, and lower palpebral conjunctiva, respectively.

FIGURE 3A

Aqueous Humor Concentrations
1 Hour after a Single Dose

Moxifloxacin
Ofloxacin

FIGURE 3B

Cornea Concentrations
1 Hour after a Single Dose
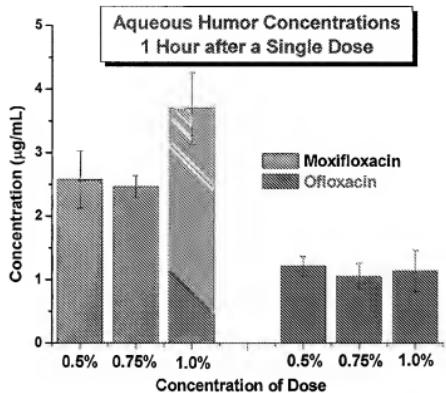
Moxifloxacin
Ofloxacin
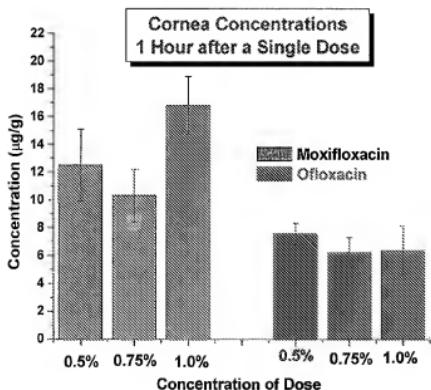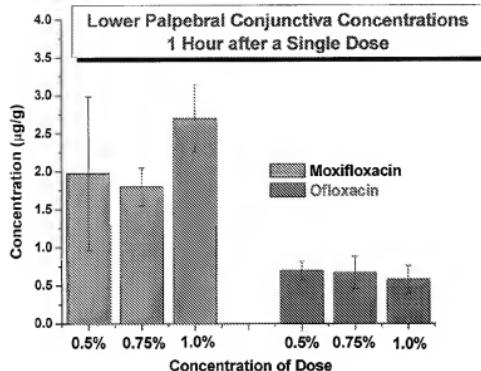
FIGURE 3C

12.   The *in vivo* results shown in Tables 3A – 3C and Figures 3A – 3C are consistent with the *ex vivo* Steady-State Model results.   At all three drug concentrations (0.5, 0.75 and 1.0 wt.%), moxifloxacin compositions exhibited significantly superior ocular penetration properties compared to ofloxacin compositions.

13.   I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine, imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Geoffrey R. Owen
Geoffrey R. Owen, Ph.D.

Date: *February 27th, 2008*

Corneal Penetration and Changes in Corneal Permeability of Moxifloxacin versus Gatifloxacin

G.R. Owen, O. Dembinska, K.R. Stout and M.K. Mendiola

Alcon Research Ltd, Fort Worth, TX

Abstract

Purpose: To compare the effects upon the cornea of moxifloxacin versus gatifloxacin by determining their corneal penetration and change in corneal epithelial permeability following exposure to fluoroquinolone solutions in ex vivo corneal perfusion models. **Methods:** The penetration studies were conducted with solutions of the fluoroquinolones (0.1mM), and the permeability studies were conducted with the commercial preparations: Vigamox™ (0.5% moxifloxacin, Alcon Laboratories, Inc.) which does not contain BAC, and Zymar™ (0.3% gatifloxacin, Allergan, Inc.) which contains 0.005% BAC. In both sets of evaluations, corneas of NZW rabbits were excised and mounted in corneal perfusion chambers according to established methods. The penetration studies were conducted with the fluoroquinolone solutions applied to the epithelial side of the cornea. The rates of accumulation of the fluoroquinolones on the endothelial side of the chamber were determined using HPLC analysis of the perfusates over 5 hours. In the permeability studies, the commercial preparations were applied to the epithelial surface for 5 min. After rinsing, corneas were exposed to carboxyfluorescein (CF) for 5 min and the perfusate collected over 2 hrs. The level of CF in the perfusate was measured by spectrophotometry. **Results:** Moxifloxacin was found to have an apparent corneal permeability coefficient of 91 x 10^{-7} cm/sec, compared to 25 x 10^{-7} cm/sec for gatifloxacin. The lag time for the appearance of moxifloxacin on the endothelial side of the cornea was 49 min compared to 99 minutes for gatifloxacin. However, permeability of the cornea to CF was 2.1 pMol/ml/min for Vigamox™ versus 3.4 pMol/ml/min for Zymar™, with peaks of accumulation of 37 pMol/ml for Vigamox™ versus 60 pMol/ml for Zymar™. **Conclusions:** The apparent corneal penetration coefficient of moxifloxacin is 3.6 x greater than gatifloxacin and its appearance on the endothelial side is 2 x faster than gatifloxacin in the absence of any penetration enhancers such as BAC. Although the drug penetration is greater, the corneal permeability to CF is 1.6 x lower after exposure to Vigamox™ compared to Zymar™, illustrating that Vigamox™ maintains better corneal integrity. This demonstrates that the superior corneal penetration of moxifloxacin is due to the inherent characteristics of the moxifloxacin molecule, and is not due to changes in the corneal epithelial intercellular (gap) junctions.

Key Words: antibiotics/antifungals/antiparasitics • anterior segment • drug toxicity/drug effects

Appendix B

TABLE B1

Ex-Vivo Corneal Perfusion Chambers
Fluoroquinolone Comparison
0.3% Solutions at pH 5.8

Fluoroquinolone	Rate (µg/min)	240 minute Accumulation (µg)	Lag Time (min)	Permeability Coefficient ($\times 10^{-7}$ cm/sec)
Moxifloxacin	1.7	325	45	85.2
	1.8	348	51	94.2
Ofloxacin	1.2	246	39	62.6
	1.1	208	48	55.6
Levofloxacin	1.1	210	41	54
	0.91	174	49	46.6

TABLE B2

Ex-Vivo Corneal Perfusion Chambers
Fluoroquinolone Comparison
0.5% Solutions at pH 5.8

Fluoroquinolone	Rate (µg/min)	240 minute Accumulation (µg)	Lag Time (min)	Permeability Coefficient ($\times 10^{-7}$ cm/sec)
Ofloxacin	2.3	441	48	70.4
	3.1	590	49	94.7
Moxifloxacin	2.4	484	42	75
	2.5	491	44	77
Levofloxacin	1.9	386	42	59.7
	1.6	308	43	47.9

TABLE B3

Ex-Vivo Corneal Perfusion Chambers
Fluoroquinolone Comparison
0.75% Solutions at pH 5.8

Fluoroquinolone	Rate (μg/min)	240 minute Accumulation (μg)	Lag Time (min)	Permeability Coefficient ($\times 10^{-7}$ cm/sec)
Ofloxacin	3.4	667	42	68.6
	3.6	703	45	74.6
Moxifloxacin	3.2	628	44	65.4
	3.6	701	46	74
Levofloxacin	2.9	571	46	60.1
	2.8	526	51	57.1

TABLE B4

Ocular Distribution of Fluoroquinolones
After Topical Dosing @ pH 5.8

Concentration	0.5 %	0.75 %	0.5 %	0.75 %
Fluoroquinolone	**Moxifloxacin**		Ofloxacin	
Tissue	Concentration (μg/g or μg/mL) Mean ± SE (n = 2)			
Aqueous Humor	**1.8 ± 0.6**	**3.8 ± 1.4**	1.0 ± 0.2	1.9 ± 0.1
Cornea	**11 ± 4**	**15 ± 6**	9 ± 3	15.9 ± 0.1
Upper Palpebral Conjunctiva	**0.6 ± 0.3**	**2.2 ± 1.2**	1.2 ± 0.5	2.4 ± 0.7
Lower Palpebral Conjunctiva	**1.5 ± 0.8**	**3.6 ± 1.7**	2.1 ± 1.4	4.0 ± 0.7
Iris- Ciliary Body	**1.1 ± 0.5**	**2.5 ± 1.1**	0.9 ± 0.2	1.7 ± 0.4

2

1 Q.   Let's take a look at the 4942 patent, which is

2 Plaintiffs' Exhibit 3. If we can go to the second page.

3   Have you reviewed this document?

4 A.   Yes.

5 Q.   Does it disclose the structure of moxifloxacin?

6 A.   Yes.

7 Q.   Let's take a look at the general formula in the '942

8 patent which is at columns one to two.

9   How many compounds are disclosed in this general

10 formula?

11 A.   Billions of compounds.

12 Q.   Do any of those compounds have a methyl group in the

13 3 position?

14 A.   Not a one.

15 Q.   Let's take a look just at column one and focus on the

16 title of the '942 patent.

17   What, if anything, would the title indicate to

18 the person of ordinary skill regarding the structure of the

19 compounds disclosed in this patent.

20 A.   They're all 3-carboxylic acids. They're all

21 quinolone, naphthyridone, are called quinolones because

22 they're in the same general classification and structure.

23 They're a quinolonecarboxylic acid and they're

24 antibacterials.

25 Q.   What would the information we discussed in the '942

1   MR. PERLMAN: I appreciate that. Thank you.

2   (Short recess taken.)

3   - - -

4   (Proceedings resumed after the short recess.)

5   THE COURT: You may continue.

6   MR. PERLMAN: Thank you, your Honor.

7 BY MR. PERLMAN:

8 Q.   Dr. Allen, I would like to turn our attention to the

9 '942 patent. All right?

10 A.   Okay.

11 Q.   Do you have a hard copy in front of you still?

12 A.   I should have.

13 Q.   I'm going to put it up on the screen, but if you have

14 a hard copy, feel free to follow along.

15   Do you have it, sir?

16 A.   Yes.

17 Q.   Okay. Now, I'm going to focus on -- you agree that

18 in Column 54 of the patent, there's a list of the numbered

19 diseases that the compounds, according to the invention, can

20 be used against?

21   Let me do it this way.

22   MR. PERLMAN: Can we put up PTX-3-30, Snap 5,

23 which is Column 54 of the '942 patent?

24 BY MR. PERLMAN:

25 Q.   And it says, Dr. Allen, at the top of Column 54,

1   examples which may be mentioned of diseases which are caused

2   by the pathogens or mixed infections mentioned and can be

3   prevented, alleviated or cured by the compounds according to

4   the invention are:

5   Do you see that?

6   A.   Yes.

7   Q.   And so what it's saying here is that the compounds

8   according to the invention can prevent, alleviate, or cure

9   what's going to be coming next, a left of diseases or

10   infections; is that correct?

11   A.   That's correct.

12   Q.   Okay. Let's go to Snap 6, please.

13   And the next thing that the patent says is, it

14   gives a list of more than four dozen kinds of diseases or

15   infections in humans.

16   Do you see that?

17   A.   Yes.

18   Q.   And would you agree with me it's a very diverse list

19   of types of infections?

20   A.   Yes, it is.

21   Q.   It includes pulmonary emphysema; right?

22   A.   Yes.

23   Q.   Cystic fibrosis; right?

24   A.   Right.

25   Q.   Burn wounds; right?

1   A.   Right.

2   Q.   Typhoid; right?

3   A.   I'm looking for typhoid.

4   Q.   Third line from the bottom?

5   A.   Yes.

6   Q.   Would you agree with me typhoid is there?

7   A.   Yes.

8   Q.   Okay. Multitude and multitude of other diseases and

9   infections in humans?

10   A.   Yes.

11   Q.   Let's go to the next snap, Snap 7, please, which is

12   the next paragraph in Column 54.

13   It says, as well as in humans, the

14   compounds, according to the invention, can also treat

15   bacterial infections in other species.

16   Do you see that?

17   A.   Yes.

18   Q.   And so not only can it treat these dozens and dozens

19   of -- not only can the compound according to the invention

20   treat these dozens and dozens of diseases in humans, they

21   can also treat a list of infections in a number of different

22   kinds of animals; correct?

23   A.   Correct.

24   Q.   Including pigs; right?

25   A.   Correct.

1   Q.   Ruminants, which would include cattle, sheep and

2   goats; right?

3   A.   Right.

4   Q.   And horses?

5   A.   Right.

6   Q.   Dogs and cats?

7   A.   Right.

8   Q.   Poultry; right?

9   A.   Right.

10   Q.   And lots of different kinds of poultry?

11   A.   Right.

12   Q.   Chickens and turkeys and quail pigeons; right?

13   A.   Right.

14   Q.   Also ornamental birds; right?

15   A.   Right.

16   Q.   And others?

17   A.   Right.

18   Q.   And for each of the kinds of animal, it lists several

19   different kinds of diseases; right?

20   A.   Right.

21   Q.   Let's look at the next paragraph of Column 54, which

22   is Snap 8.

23   It says, in addition to the humans and the

24   animals we just looked at, the compounds according to the

25   invention can also be used to prevent, alleviate or cure

1   certain bacterial diseases in the rearing and keeping of

2   fish.

3   Do you see that?

4   A.   Right.

5   Q.   And not just stock fish; right? Also ornamental

6   fish; right?

7   A.   Right.

8   Q.   And the patent lists some additional pathogens that

9   are peculiar to fish.

10   Do you see that?

11   A.   Right.

12   Q.   And as I understand your testimony on direct, you

13   believe that the '942 patent lists moxifloxacin as the

14   preferred compound; is that correct?

15   A.   Yes.

16   Q.   And you said that moxifloxacin is a preferred example

17   in the patent; is that right?

18   A.   Moxifloxacin is the one that's listed in Claim 1 as

19   the preferred compound, yes.

20   Q.   And did you also say it was a preferred example?

21   A.   I don't -- I don't recall one way or the other.

22   Q.   Okay. And it would be your view that the person of

23   ordinary skill in the art would read this patent as saying

24   that moxifloxacin could treat every one of the diseases and

25   infections we just looked at?

1   A.   I would have no reason to doubt the -- the content of

2   the patent, yes.

3   Q.   All right. So your opinion would be that because

4   moxifloxacin is a preferred compound, the person of ordinary

5   skill would read the patent to say that moxifloxacin could

6   treat each and every one of the diseases and infections

7   in humans, animals and fish that we just looked at;

8   correct?

9   A.   That's what it says, yes.

10   Q.   And I take it, it would be your view that the person

11   of ordinary skill would read this patent to say that the

12   compounds that the patent identifies as the preferred

13   compounds could be used to treat all these different

14   diseases and infections. Would that be fair?

15   A.   Again, there is no reason to doubt what is in the

16   patent.

17   Q.   So it would be your view that the person of ordinary

18   skill would read this patent to be saying that the compounds

19   that the '942 patent identifies as the preferred compounds

20   could treat all the infections that we just saw listed in

21   column 54; correct?

22   A.   As I said, there is no reason to doubt what is in the

23   patent.

24   Q.   And, Dr. Allen, would you answer my question. The

25   person of ordinary skill would read the patent to be saying

1 that the compounds that the '942 patent identifies as the

2 preferred compounds could treat all of the diseases and

3 infections listed in column 54; correct?

4 A.   They would accept what the patent says, yes.

5 Q.   All the diseases in humans; right? That the

6 preferred compounds could treat all the diseases in humans?

7 A.   It talks about humans and animals.

8 Q.   So both humans and animals and fish; right?

9 A.   Yes.

10   MR. PERLMAN: Could I have a moment, Your Honor?

11   THE COURT: Yes.

12   (Pause.)

13   MR. PERLMAN: Thank you, Your Honor.

14   THE COURT: All right.

15   MR. PERLMAN: Could I see Plaintiffs' Exhibit 3,

16 starting at column 2, line 29?

17   Then if could we just start about midway down

18 the page. Do you see where it says preferred compounds are

19 those of Formula I, on the right side at line 29? And just

20 highlight the whole page there all the way down.

21 BY MR. PERLMAN:

22 Q.   Do you see, Dr. Allen, in column two the patent

23 identifies particular compounds and preferred compounds?

24 A.   And.

25 Q.   And it gives --

1 Q.   You certainly can't name a compound that could treat

2 all those diseases?

3 A.   No.

4   MR. PERLMAN: Let's go to column 56 of the

5 patent, please. And the fourth full paragraph, starting at

6 line 19, can you blow that up, please?

7 BY MR. PERLMAN:

8 Q.   Dr. Allen, I have blown up a paragraph in column 56

9 where it says the formulations mentioned can be used on

10 humans and animals, et cetera. Do you see that?

11 A.   Yes.

12 Q.   And you testified on direct I believe about this

13 paragraph?

14 A.   I believe so.

15 Q.   And you pointed out that buried in the middle of that

16 paragraph is the word "ophthalmological." Do you see that?

17 A.   Yes.

18 Q.   Would you agree with me this is a list of numbers of

19 very diverse kind of formulations?

20 A.   Yes.

21 Q.   And it would be your view that the person of ordinary

22 skill would read the '942 patent to be saying that the

23 compounds of the invention could each be used in each of

24 these formulations?

25 A.   That's what the patent says, yes.

Q.   Certainly, the compounds that are identified as the
preferred compounds in the patent could be used in each one
of these formulations; correct?

A.   That's what the patent says, yes.

Q.   And that is how the person of ordinary skill would
read it?

A.   Yes, would have no reason to doubt what the patent
says.

Q.   Would you agree with me that the person of ordinary
skill would not think that all the compounds within this
patent have been tested in every one of these different
kinds of formulations by the time the patent was drafted?

A.   I can't really evaluate what all the scientists did,
just what is stated in the patent.

Q.   Well, is it your view that the person of ordinary
skill in the art would believe that the compounds, according
to the invention in this patent, had each been tested in
each of these formulations prior to this paragraph's
inclusion in the patent?

A.   Again, that is what the patent says.

Q.   The patent says they've all been tested in all these
formulations?

A.   No, it says they can be used.

Q.   And my question, Dr. Allen, is from that phrase "can
be used," would the person of ordinary skill understand this

1   that any compound within the scope of this patent had been

2   tested in humans; correct?

3   A.   From any presented data, that's true.

4   Q.   And would you agree with me that there is no way to

5   tell, from the information in the '942 patent, whether any

6   particular compound, formulated in any particular way, would

7   be effective for the treatment of infectious diseases until

8   you formulated it, tested it, saw if it worked?

9   A.   Bottom line, that's true.

10   MR. PERLMAN: Can we put up PTX-3-30, snap 11?

11 BY MR. PERLMAN:

12 Q.   Dr. Allen, I put up on the screen, column 54, lines

13 47 through 52 of the '942 patent. This is a paragraph you

14 talked about on direct; right?

15 A.   Yes.

16 Q.   And it says: The present invention includes

17 pharmaceutical formulations which contains, in addition to

18 nontoxic, inert pharmaceutical suitable excipients, one or

19 more compounds according to the invention or consists of one

20 or more active compounds according to the invention and

21 processes for the preparation of these formulations.

22   Do you see that?

23 A.   Yes.

24 Q.   And the compounds that are being referred to there

25 are in Formula I of the patent; correct?

1   A.   Okay. Could you say that one more time?

2   Q.   When it says, "compounds according to the invention,"

3   what it's referring to is what we talked about earlier were

4   the compounds accord to the invention, those in the Formula

5   I; correct?

6   A.   Let me go through that again, real quick.

7   Okay. This is stating that you have a

8   pharmaceutical formulation which contains compounds

9   according to the invention in addition to the excipients and

10   processes for the preparation of the formulations.

11   Q.   Right.

12   A.   What was your question?

13   Q.   My question is that the compounds according to the

14   invention that are referred to here are the same compounds

15   according to the invention we just talked about, the ones

16   that are in Formula 1; right?

17   A.   I believe that's true.

18   MR. PERLMAN: Could I have Defendant's Exhibit

19   4011, please? This is a demonstrative that Mr. Gagala

20   showed you on direct.

21   Can we blow up the upper left box, please?

22   BY MR. PERLMAN:

23   Q.   And do you see where you cite in this demonstrative

24   to Column 55, lines 1 through 5?

25   A.   Yes.

1 in order to overcome the resistance that we were seeing in

2 the fluoroquinolone class of antibiotics, that we had to go

3 into a different family with a different mechanism of action

4 against these important bacterial pathogens. And the

5 oxazolidinone were one group in which the antibiotic

6 linezolid, for example, was an antibiotic we were looking at

7 could potentially provide this advantage.

8   MR. GENDERSON: Your Honor, we offer Exhibit

9 1099 into evidence.

10   MR. ROBINSON: No objection.

11   THE COURT: Thank you.

12   (Exhibit No. 1099 was received into evidence.)

13 BY MR. GENDERSON:

14 Q.   If we can go back to the Demonstrative 2019.

15   Doctor, sticking with the developing

16 resistance, could the use of a new quinolone that was less

17 active than the existing therapies affect the development of

18 resistance?

19 A.   If you had a quinolone that was less active, then it

20 would be easier for bacterias to become resistant not just

21 to that particular quinolone, but to the whole class. And

22 we knew that that was a real problem with the

23 fluoroquinolones. That mechanism of resistance was very,

24 very worrisome.

25 Q.   Were scientists looking for new compounds that

1   against a key pathogen were less active than ciprofloxacin?

2   A.   No.

3   Q.   Would the -- I'm sorry. Let's finish going through

4   and then I will go back to the top of the list. The last

5   bullet point is, as safe as current therapies?

6   A.   Yes.

7   Q.   Can you explain why that was important?

8   A.   It was extremely important. Again, we're looking at

9   normal patients who are going to be undergoing cataract

10 surgery, who are going to be undergoing LASIK surgery, and

11 we needed to put in their eyes an antibiotic to prevent an

12 infection.

13   So the last thing we wanted to do was to

14 use an antibiotic that would not be safe inasmuch as we

15 could, and this was a concern from prior experiences we had

16 with antibiotics, that we did not want to do any harm to a

17 patient in attempts of preventing an infection.

18 Q.   How likely was it in cataract or LASIK surgery that

19 without any prophylactic treatment at all, the patient would

20 end up getting an infection?

21 A.   Well, the state of the art was to use a prophylactic

22 antibiotic to prevent an infection, and even though the

23 incidence of infections was low, for example, in cataract

24 surgery, maybe one in 1500 to 2,000 cases, and in LASIK

25 surgery, maybe one in 500 to 2,000 cases, it was hard at

1   left the tissue quickly, it wouldn't be around for when the

2   organism starts to grow and hence wouldn't be as

3   susceptible. So you needed a drug that would get there

4   quickly but stay there for a prolonged period of time.

5   Q.   You mentioned mycobacterium chelonei and

6   mycobacterium fortuitum. Are they the same as mycobacterium

7   tuberculosis?

8   A.   No, mycobacterium tuberculosis is what we commonly

9   talk about when we say someone has tuberculosis, and this

10   is principally an infection of the chest. This is a chronic

11   respiratory tract infection that gets worse over time.

12   Mycobacterium tuberculosis is not an important ophthalmic

13   pathogen. It is not an important pathogen in key ocular

14   tissues within the cornea and also as a cause of

15   endophthalmitis.

16   Q.   Can one predict a compound's activity against

17   mycobacterium chelonei and mycobacterium fortuitum on the

18   basis of its activity against mycobacterium tuberculosis?

19   A.   No.

20   Q.   Now, Dr. Zhanel, you said there are two factors,

21   pharmacokinetics and pharmacodynamics. Can you explain what

22   you mean by pharmacodynamics?

23   A.   Pharmacodynamics is the fun stuff. Pharmacodynamics

24   is once the drug gets to the site of infection, how is it

25   able to interact with and kill the pathogen. And what we

1   know is that the drug has to kill the pathogen as quickly as

2   possible, because two things will occur. If you kill the

3   pathogen, you are going to cure the patient quickly and

4   their signs and symptoms will go away.

5   But perhaps more importantly, if you kill the

6   pathogen you are less likely to have a resistant strain

7   develop. We always like to teach students that dead bugs

8   don't mutate. So your goal is to kill it. And that way it

9   won't mutate into a resistant strain.

10   If you don't kill it, you wound it, all it's

11   going to do is become resistant to not just your drug but

12   all the drugs within that class. And this was a critical

13   issue occurring with the fluoroquinolones in September 1998.

14   Q.   Dr. Zhanel, in the first instance, how were the

15   pharmacokinetics of the compound assessed?

16   A.   Pharmacokinetic testing always starts in the

17   test-tube, doing MIC testing.

18   Q.   Have you conducted MIC tests?

19   A.   I have tried to add up how much, and the answer is

20   yes. And I think we have done well over 100,000 MIC tests.

21   Q.   Have you compared activity of compounds using MIC

22   tests?

23   A.   Yes.

24   Q.   How often do you do that?

25   A.   I do every single day. I have been doing that for

1   an order of magnitude?

2   A.   No.

3   Q.   Now, Dr. Zhanel, noted that a few of the compounds

4   here are highlighted in yellow, BAY 12-8039. Is that

5   moxifloxacin?

6   A.   Yes.

7   Q.   And we've also highlighted ciprofloxacin. Is

8   ofloxacin on the list?

9   A.   No.

10   Q.   Why not?

11   A.   It's not the market standard. Ciprofloxacin was the

12   market standard so every quinolone wanted to compare itself

13   to ciprofloxacin.

14   Q.   And what is the difference in pseudomonas activity

15   between ciprofloxacin and BAY 12-8039, moxifloxacin?

16   A.   It's eightfold. But, first of all, let me apologize

17   for the colors. This is my fault. I thought pink would

18   look better.

19   But what I'm showing you there is moxifloxacin

20   against pseudomonas, the MIC is 2. And for ciprofloxacin,

21   the MIC is .25. That is called an eightfold difference.

22   And that is a significant difference.

23   Q.   Would anyone skilled in the art of microbiology

24   conclude that an eightfold difference in activity against

25   pseudomonas is not significant?

1   A.   No, this is hugely significant.

2   Q.   What about the activity against MSSA and MRSA?

3   A.   These are both Staph. aureus. What MSSA stands for

4   is methicillin-susceptible Staph. aureus. MSRA stand for

5   methicillin-resistant Staph. aureus. So these are two

6   different favors of Staph. aureus. The MSRA is the really

7   scary one. And what you see there is that moxifloxacin is

8   more active than ciprofloxacin against both MSSA and MRSA.

9   And this is again significant because these are large

10   differences.

11   Q.   Dr. Zhanel, I see next to the names of the pathogens,

12   for example, by Pseudomonas aeruginosa it says 15, MSSA 54,

13   MRSA 20. What do those numbers mean?

14   A.   It represents the number of Pseudomonas aeruginosa or

15   Staph. aureus that were studied. The larger the number, the

16   better. Microbiologist like to see data on large amount of

17   organisms. So, for example, if there were only two

18   pseudomonas studied, it wouldn't give me as much weighting.

19   I wouldn't put as much emphasis or impact on that data as if

20   I saw 15 strains studied.

21   Q.   What is that?

22   A.   The more data you study, the more organisms you study

23   the better, because you get a feeling this is the actual

24   result that you are going to get in clinical practice where

25   you could encounter many, many different pseudomonas, not

1 aeruginosa, but its activity is looking good for other

2 infections, principally community acquired respiratory tract

3 infections and potentially other infections, such as

4 genitourinary, which are things like kidney, bladder

5 infections, intra-abdominal infections, skin and skin

6 structure infections. These community acquired infections

7 are infections where pseudomonas is not a problem.

8   So these would be potential targeted

9 infections to go after with moxifloxacin because pseudomonas

10 is not at issue, but moxifloxacin did cover pathogens

11 involved in those infections.

12 Q.   Doctor Zhanel, on the basis of the available data

13 provided in the poster PTX-1098 and all the other references

14 that you reviewed, would a person of ordinary skill in the

15 art have had interest in making a top all ophthalmic

16 formulation containing moxifloxacin?

17 A.   No.

18 Q.   Why not?

19 A.   Because it didn't have the properties that I talked

20 about. Compared to the standard ophthalmic fluoroquinolone

21 compositions in September 1998, moxifloxacin, although being

22 more active against the gram-positive Staph aureus, it was

23 significantly less active against pseudomonas aeruginosa,

24 and pseudomonas aeruginosa was a critical ophthalmic

25 pathogen, and if you didn't cover it, you had no hope of

1 being an ophthalmic composition antimicrobial.

2 Q.   Couldn't a person of ordinary skill in the art have

3 been interested in moxifloxacin on the basis of its improved

4 Staph aureus activity despite its diminished pseudomonas

5 activity?

6 A.   Not for ophthalmic infections. A person of ordinary

7 skill would know that if you gain on the one hand Staph

8 aureus, but lose on the other hand with pseudomonas

9 aeruginosa, that you are taking one step forward and two

10 steps back.

11   More importantly, there were options for Staph

12 aureus for topical ophthalmic compositions. There were

13 other agents, other classes, that had activity against Staph

14 aureus, but for pseudomonas aeruginosa, very limited

15 therapies were available, and a person of ordinary skill

16 would know that if I cannot kill the most important

17 intraocular pathogen pseudomonas, all I'm going to do is

18 predispose patients to clinically failing and, perhaps

19 more importantly, if I don't kill the organism, I'm going to

20 drive quinolone resistance through the roof. I'm going to

21 take the most important problem in ocular infectious

22 diseases in September 1998, and by using a drug that

23 won't kill pseudomonas, I'm going to make the problem

24 worse.

25 Q.   Dr. Zhanel, you mentioned other potential treatments

1   for staphylococcus aureus. What would those have been?

2   A.   The other treatments available in September 1998

3   for Staph aureus ophthalmic infections could have included

4   the macrolides, could have included the aminoglycosides,

5   could have included potentially sulfonamides, could have

6   included combination therapies, such as polymixin and

7   trimethoprim.

8   There were other classes that could be used for

9   Staph aureus.

10   Q.   Couldn't moxifloxacin have been useful in treating

11   ophthalmic infections that someone knew was caused by

12   staphylococcus aureus?

13   A.   No.

14   Q.   Why not?

15   A.   And the reason is, patients don't come into a clinic

16   or the Emergency Room or right to a medical ward or to the

17   Intensive Care community with a label on their forehead

18   that says I have pseudomonas endophthalmitis. Patients

19   come in with an infection. They come in with signs and

20   symptoms. What we do, we start therapy empirically.

21   Empirically means we don't know what the pathogen is, and

22   we initiate broad spectrum antimicrobial activity that

23   covers everything.

24   So if you have a potential ocular infection

25   that you are treating, or preventing, you have to start an

1   antimicrobial that's going to cover the different

2   possibilities, because it will take you days before you know

3   what the pathogen is, and frequently we never find out what

4   the pathogen is. You could not afford to be wrong in

5   seriously ill patients with invasive ocular infections.

6   Q.   Would a person of ordinary skill in the art have

7   considered moxifloxacin based on its improved activity

8   against staphylococcus useful as a prophylaxis?

9   A.   No.

10   Q.   Why not?

11   A.   For the same reasons that I described . When you are

12   preventing ocular infections post-surgery, you don't know if

13   that patient may become infected with a Staph aureus or a

14   pseudomonas aeruginosa or a mycobacterium chelonae. You

15   don't know what the organism may be that will cause that

16   post-surgical infection, so you can't be wrong. You start

17   broad spectrum antimicrobial preventive therapy and the

18   topical composition must be broad spectrum so it can prevent

19   the myriad of different organisms that could cause that

20   post-surgical infection.

21   Q.   You've been using the term broad spectrum. Does the

22   use of that term depend on the particular tissue where the

23   infection was being used?

24   A.   Certainly.

25   Q.   Why is that?

A.   In different tissues, different infections and different pathogens target different tissues. So what may be considered the appropriate broad spectrum therapy in one tissue, we will use for different tissue, we would say a different broad spectrum therapy is needed for a different tissue.

Q.   You've mentioned the idea of pseudomonas aeruginosa resistance several times today. Was Pseudomonas aeruginosa resistance to ciprofloxacin a significant concern to the person of ordinary skill in the art in September 1998?

A.   Yes, it was. In the mid-90's, quinolone, that is ciprofloxacin, ofloxacin resistant pseudomonas, was as I said, in the systemic field, in infections of the blood, for example, infections in Intensive Care Units. But it was already being published in the ophthalmological literature even though it wasn't occurring in ophthalmology or in ocular isolates.

                    The ophthalmologists were worried because they knew it was just a matter of time before it came into ophthalmology. And then, in September 1998, it was clear that the ophthalmologic barrier was broken, and what happened is exactly what ophthalmologists knew would happen, is that quinolone resistant Pseudomonas aeruginosa was there. It was leading to problems in terms of treatment and prophylaxis of ocular infections, and this was a major

1 issue in September '98 to ciprofloxacin and all the

2 quinolones.

3 Q.   Was this a significant problem back in 1993?

4 A.   No.   In the late 80's and early 90's, in 1993, when

5 ofloxacin topical ophthalmic came to the market, quinolone

6 pseudomonas was not a huge issue, and certainly not an issue

7 in ophthalmology.   However, in the mid-90's, it started to

8 escalate, started to grow rapidly, and by September '98, it

9 was an issue not just systemically, but also in the

10 ophthalmic literature.

11 Q.   Let's take a look at Plaintiffs' Exhibit 184-D, which

12 is from the Archives of Ophthalmology.

13   Let's go to Snap D1.   The title of this paper is

14 Scleral Buckle Infection with Ciprofloxacin Resistant

15 Pseudomonas Aeruginosa.

16   And I've blown up the first paragraph.

17   Let's go through that paragraph sentence by

18 sentence.   Let's go to the next Snap.

19   The first sentence says Knauff, et al showed a

20 statistically significant increase in ciprofloxacin

21 resistant systemic isolates from 1988 to 1983.

22   What does that mean?

23 A.   I think you're incorrect.   It was 1993.

24 Q.   Excuse me.

25 A.   But what Knauff is saying, he and his colleagues were

assessing quinolone resistance in gram-negative bacilli,

including pseudomonas aeruginosa, to the fluoroquinolones

over a period of '88 to 1993.

And what they saw was that quinolone

resistance was increasing in systemic isolates, whether they

were gram positives, gram negatives, but it included

pseudomonas aeruginosa.

So although these were not yet ocular isolates,

they were systemic isolates, it was being published in the

ocular journals, because everybody knew that it was just a

matter of time before this came in to important ocular

infections.

Q.   Have you reviewed the Knauff paper?

A.   Yes.

Q.   Let's take a look at the next Snap. It says all of

these systemic isolates are also common ocular pathogens.

What does that mean?

A.   What it means is that there's nothing magical between

isolates that cause systemic infections, such as hospital

acquired pneumonia, and isolates that cause infections in

ocular tissue, even though these isolates are different and

we always like to see literature on ocular isolates.

If resistance occurs in systemic isolates, we

know that eventually, resistance will occur in ocular

pathogens. So it's telling people that we're going to be in

1 trouble very shortly.

2 Q.   Let's take a look at the third sentence, which reads,

3 if the present trend continues, widespread ciprofloxacin

4 resistance among the common ocular isolates may occur in the

5 near future.

6   How would a person of ordinary skill in the art

7 have interpreted that sentence?

8 A.   Person of ordinary skill would look at that sentence

9 and say that it is just a matter of time before a quinolone

10 resistance is a huge problem in patients with ocular

11 infections.

12 Q.   And what is the present trend that is being

13 referenced in that sentence?

14 A.   Present trend is widespread ciprofloxacin resistance,

15 including Pseudomonas aeruginosa.

16 Q.   Dr. Zhanel, did ocular resistance to pseudomonas

17 occur as predicted here?

18 A.   Yes, it did.

19 Q.   Let's take a look -- actually, let's move Exhibit

20 184D. We offer that exhibit.

21   MR. HEFNER: No objection.

22   THE COURT: Thank you.

23   (Plaintiffs' Exhibit No 184D was received into

24 evidence.)

25 BY MR. BERL:

1   Q.   Dr. Zhanel, let's take a look at Plaintiffs' Exhibit

2   189, which is entitled Emerging Ciprofloxacin Resistant

3   Pseudomonas Aeruginosa, by Chaudhry, et al. And let's take

4   a look at Snap 1, which is Figure 1 of the article.

5   Can you explain what Figure 1 of the article

6   shows?

7   A.   Figure 1 shows the susceptibility to ciprofloxacin of

8   ocular Pseudomonas aeruginosa isolates. And to make it

9   clear, susceptible is the inverse of resistance. So as

10   susceptibility goes down, resistance goes up.

11   Q.   A nonsusceptible strain?

12   A.   Is a resistant strain. Exactly.

13   Q.   And what does this comparison show in Figure 1?

14   A.   This comparison shows that between the study years of

15   1991 to 1994, ciprofloxacin resistant pseudomonas aeruginosa

16   ocular isolates was low. Susceptibility was close to a

17   hundred percent.

18   However, things changed quite dramatically to

19   '95-1998, where susceptibility went down from a hundred to

20   approximately 95, 94 percent, but it basically told us that

21   resistance went from essentially zero to about five percent.

22   And a person of ordinary skill would look at these data and

23   say, it's here. We've got a problem.

24   Q.   Was there other literature that reflected this growth

25   in pseudomonas resistance --

1 A.   Yes.

2 Q.   -- to ciprofloxacin in the 1990's?

3 A.   Yes.

4 Q.   Dr. Zhanel, in view of the problem of emerging

5 resistance to ciprofloxacin in the eye, what would a person

6 of ordinary skill in the art interested in finding a more

7 effective therapy have done in September 1998?

8 A.   A person of ordinary skill looking to find new,

9 better therapies would have done one of two things. The

10 first one would have involved trying to find new classes of

11 antimicrobials and not simply focusing on the quinolones.

12 Q.   Why would they have done that?

13 A.   Their assumption would be that while I've got

14 quinolone resistance to pseudomonas and staph, and just

15 working on more and more quinolones is not going to get me

16 anywhere, I'm just going to have more quinolone resistance,

17 because it's a class effect, so they would look to

18 developing new classes of antimicrobials, agents that work

19 completely differently than the quinolones.

20   The rationale would be if I can develop a

21 compound or ocular ophthalmic infections that has a

22 different mechanism of action, I will actually be able to

23 kill pathogens resistant to other classes, like the

24 quinolones, so I will be able to kill these quinolone

25 resistant strains.

1 Q.   Can you explain how resistance occurs?

2 A.   Resistance occurs when you use an antimicrobial, you

3 don't kill the pathogen. The pathogen has an opportunity to

4 mutate and change the binding cites of where the drug is

5 working, and you develop resistance not only to that drug,

6 but drugs within that class. And this is what we worry

7 about, is that if you are using a drug that is weaker than

8 other agents in the class, you'll be less likely to kill the

9 pathogen, more likely to allow resistance to occur, so by

10 using the weakest drug in the class, you drive resistance to

11 not just this drug, but all of the drugs in that class. And

12 this is a huge issue.

13 Q.   Was that a commonly understood principle by September

14 1998?

15 A.   Absolutely, especially with the quinolones and

16 pseudomonas aeruginosa.

17 Q.   Were there any well-known examples of that happening?

18 A.   Yes, there were.

19 Q.   And can you provide one?

20 A.   An important example was a large Medical Center in

21 Chicago, where the investigators had ciprofloxacin on the

22 hospital formulary. And resistance was quite low with

23 agents such as pseudomonas aeruginosa. Things were going

24 quite well with ciprofloxacin. But then the hospital wanted

25 to save some money. They got a bargain. They got -- they

1 took cipro off the formulary and started to use ofloxacin,

2 because it was cheaper. And very quickly, ofloxacin

3 resistant pseudomonas aeruginosa started to increase

4 dramatically in this Medical Center.

5 Q.   When did this happen, approximately?

6 A.   This was happening in the mid-90's. And this was

7 well documented by a world class research group, colleagues

8 of mine, and they were presenting these data at all the key

9 meetings, saying, look. We drove ofloxacin resistant

10 Pseudomonas aeruginosa through the roof at our hospital, so

11 we all said to them, you know what? Go back to cipro.

12 Q.   What happened then?

13 A.   So they went back to cipro, and unfortunately, it was

14 difficult to resurrect the problem, because once you have

15 ofloxacin resistant pseudomonas aeruginosa, you had

16 ciprofloxacin resistant pseudomonas aeruginosa, you had

17 quinolone resistant pseudomonas aeruginosa.

18   So they ended up with a huge problem, but it

19 taught everyone that if you start messing around with the

20 most important ocular pathogen, like pseudomonas aeruginosa,

21 and toying with it, with a drug like ofloxacin, instead of

22 killing it, you're going to create resistance to not just

23 ofloxacin, but to the whole class of quinolones.

24 Q.   Dr. Zhanel, you talked about looking at other classes

25 of antibiotics.

1   Were skilled artisans in 1998, in fact, looking

2   at other classes as a result of the quinolone resistance

3   problems?

4   A.   Yes.

5   Q.   What were some examples?

6   A.   There were numerous antimicrobial classes that were

7   being investigated. People were looking at the carbapenems

8   as a potential solution to quinolone resistant Pseudomonas

9   aeruginosa. Investigators were looking at the fourth and

10 the fifth generation cephalosporins that had activity

11 against both MRS Staph aureus and Pseudomonas aeruginosa.

12   Investigators were also looking at

13 completely different classes, agents such as the

14 oxazolidinones, agents such as the glycolipopeptides and

15 others. People were being very ingenious in asking

16 themselves what can we do to combat this emerging issue and

17 they were thinking out of the box and trying to come up with

18 new agents.

19 Q.   And when you say thinking out of the box, are you

20 talking about a completely new class, like the

21 oxazolidinones?

22 A.   That's right. Basically saying, you know what, let's

23 not just be playing around with the quinolones. Let's think

24 of other classes. Let's discover new mechanisms of action.

25 Let's try something different. Use agents that had never

1   been used, potentially, to treat these resistant

2   pseudomonas.

3   Q.   Are these classes that you mentioned as toxic as

4   quinolones?

5   A.   No.   The carbapenems, and there are examples, are

6   extremely safe compounds. The cephalosporins have been

7   around for a very long time. They are very, very safe

8   compound. The oxazolidinones are new. We don't have as

9   much information, but they look safe and definitely much

10   safer than the quinolones. And the glycolipopeptides are

11   looking quite good as well.

12   Q.   Now, Dr. Zhanel, if one had chosen to stay within the

13   class of quinolones, what would have been the requirement

14   for a person of ordinary skill in the art in terms of

15   pseudomonas?

16   A.   If you were going to stay in the quinolone area, your

17   criteria would be that you should not be less active than

18   ciprofloxacin for pseudomonas. Ideally, you would be as

19   active, and ideally more active than ciprofloxacin, and that

20   you would gain on the gram-positive Staph aureus side. That

21   was the standard.

22   Q.   What would a person of ordinary skill in the art in

23   September 1998 have concluded about the effect of using

24   moxifloxacin to treat pseudomonas infections in the eye in

25   September 1998?

1 A.   A person of ordinary skill in September '98 would

2 have concluded that if moxifloxacin ophthalmic solution

3 would have been used to treat pseudomonas aeruginosa eye

4 infections, that you would take the most important problem

5 in ocular infectious diseases at that time and make it

6 worse.   That is, because moxifloxacin wasn't active against

7 pseudomonas aeruginosa, the expectation would have been that

8 you would have clinical failures, and that you would have

9 resistance develop and you would drive quinolone resistance

10 pseudomonas aeruginosa very quickly.

11 Q.   Dr. Zhanel, in the documents you've reviewed in

12 connection with this case, have you found any evidence

13 that moxifloxacin would be useful against pseudomonas

14 resistant -- against ciprofloxacin resistant pseudomonas

15 strains as of September 1998?

16 A.   No.

17 Q.   We saw earlier that ofloxacin, the active ingredient

18 in Ocuflox, is less active than ciprofloxacin against

19 pseudomonas.

20   How do you explain that, given your view?

21 A.   My view is that back in the 80's, when ciprofloxacin

22 ophthalmic solution and ofloxacin -- sorry. I may have --

23 both ofloxacin and ciprofloxacin ophthalmic solutions were

24 being developed, there was an urgent need for a new class of

25 antimicrobials that could be used ocularly to treat

1 there and assess are the alternatives more potentially

2 effective than ciprofloxacin and how does their toxicity

3 occur? So you would have to look at all the alternatives

4 and ask yourself will they be better than cipro? Will they

5 be as safe or safer than ciprofloxacin?

6 Q.   And in the context of the existing and alternative

7 therapies and the expected use of the compounds, how would a

8 person of ordinary skill in the art assess the benefit

9 associated with the use of moxifloxacin ophthalmically in

10 September 1998?

11 A.   In September '98, a person of ordinary skill would

12 have compared moxifloxacin to ciprofloxacin and said, well,

13 I gain on the gram-positive Staph. aureus side but I'm

14 losing on the most important pathogen, Pseudomonas

15 aeruginosa. I do not think that I'm going to get any added

16 value compared to ciprofloxacin. In fact, I think I may

17 actually lose on the pseudomonas side. I may have clinical

18 failures with moxifloxacin for pseudomonal eye infections

19 and I will likely drive resistance to entire class of

20 quinolones so it would have looked upon as likely being less

21 effective.

22 Q.   Now I would like to look at the other half of the

23 equation, the risk for toxicity. Was there a general trend

24 with regard to the toxicity of quinolones?

25 A.   Yes, there was.

1 the scope of the '942 patent.

2 Q.   Would a person of ordinary skill in the art

3 understand Column 53 to disclose that each of the billions

4 of compounds, according to the invention, have a low

5 toxicity and exhibit a broad antibacterial spectrum?

6 A.   No.

7 Q.   Would a person of ordinary skill in the art

8 understand the '942 patent to disclose that any one of the

9 compounds of the invention has a low toxicity and exhibits a

10 broad antibacterial spectrum?

11 A.   No.

12 Q.   Dr. Zhanel, Dr. Allen testified that the '942 patent

13 discloses moxifloxacin as the most preferred compound in the

14 '942 patent. Do you agree?

15 A.   No.

16 Q.   Why not?

17 A.   Because the preferred compounds represent millions

18 and billions of compounds.

19 Q.   Let's take a look at Column 2 of the '942 patent

20 where it discloses preferred compounds. Is moxifloxacin one

21 of the preferred compounds?

22 A.   Yes.

23 Q.   How many compounds are disclosed within the '942

24 patent's preferred compounds?

25 A.   Billions.

1   Q.   Are there any compounds in the '942 patent that are
2   disclosed as particularly preferred?

3   A.   Yes.

4   Q.   Let's take a look at Columns 3 and 4 of the '942
5   patent where it discloses particularly preferred compounds.
6   How many such compounds are there, Dr. Zhanel?

7   A.   The particularly preferred compounds represents over
8   a million compounds.

9   Q.   Is moxifloxacin one of those over a million
10   compounds?

11   A.   No.

12   Q.   Now, I noticed that position eight is indicated by A
13   here. And it says, A represents N or C-R8 and R8 represents
14   hydrogen, flourine, chlorine, et cetera. Are hydrogen,
15   flourine and chlorine understood by persons of skill to be
16   eight position substituents for quinolones?

17   A.   Yes.

18   Q.   Are they stood to be one position substituents?

19   A.   No.

20   Q.   Dr. Zhanel, let's now take a look at Column 54 of the
21   '942 patent which discloses various diseases and infections.
22   First of all, was there any compound known in 1998 that
23   could treat or prevent all of the diseases listed in the
24   '942 patent?

25   A.   No.

1 Q.   Would a person of ordinary skill in the art

2 understand that all of the billions of compounds of the

3 invention of the '942 patent could be used to treat every

4 one of these inventions?

5 A.   No, they wouldn't.

6 Q.   Why not?

7 A.   There has never been a compound that has ever been

8 invented that could treat all of these infections. A person

9 of ordinary skill would look at these statements to be

10 general and to potentially say there may be a compound that

11 could treat one or more of these infections. That they

12 certainly wouldn't take it that each one of the compounds or

13 one particular compound could treat something that no other

14 compound has been able to do in the past.

15 Q.   If a person of ordinary skill in the art wanted to

16 know whether any particular compound could treat any one

17 particular infection listed here, what would such a person

18 do?

19 A.   You would either go to the data or you would have to

20 study the compound.

21 Q.   Is there any data presented in the '942 patent?

22 A.   There is data in the '942 patent.

23   MR. BERL: Let's take a look at that data in

24 Column 58 of the '942 patent.

25 BY MR. BERL:

1 Q.   Have you reviewed this data, Dr. Zhanel?

2 A.   Yes.

3 Q.   What kind of data is this?

4 A.   This is microbiology data, MIC data.

5 Q.   Is there any data presented here for the compound

6 moxifloxacin?

7 A.   No.

8 Q.   Is there any any data here for any of the compounds

9 regarding activity against Pseudomonas aeruginosa?

10 A.   No.

11 Q.   What would that disclose to a person of ordinary

12 skill in the art about the '942 patent's disclosure?

13 A.   It would tell a person of ordinary skill that

14 there is no data in the entire patent that focuses at all

15 on ocular infections or at all focusing someone on a

16 moxifloxacin ophthalmic composition. There is no data

17 there.

18 Q.   Dr. Zhanel, would a person of ordinary skill in the

19 art focus on what compounds have data in a patent or what

20 compounds are claimed?

21 A.   Persons of ordinary skill go to the data. They're

22 not patent lawyers. They don't know exactly why things are

23 claimed but they do know why things go into tables with data

24 and they would go to where the data is and that is what they

25 would focus on.

1   Q.   Dr. Zhanel, are there compounds in the '942 patent

2   that are disclosed as having been synthesized by the

3   inventors?

4   A.   Yes.

5   MR. BERL: Let's take a look at one of those

6   examples in Column 77 of the '942 patent which has Example

7   4.

8   BY MR. BERL:

9   Q.   How would a person of ordinary skill in the art know

10   that this compound had been prepared?

11   A.   The synthetic chemical structure is depicted as well

12   as there are some characteristics described in terms of

13   yield, in terms of melting point, and there are over 50 such

14   examples listed in the '942 patent. A person of ordinary

15   skill would take from these examples that these examples

16   have been synthesized, they have been at least characterized

17   in part and they would be available for testing and further

18   study.

19   Q.   Is moxifloxacin one of the 50-or-so examples that are

20   disclosed in the '942 patent as having been synthesized?

21   A.   No.

22   Q.   What would a person of ordinary skill in the art

23   conclude as to whether moxifloxacin had been synthesized

24   when the '942 patent application was filed?

25   A.   A person of ordinary skill would have concluded it

1 had not been synthesized as none of the examples showing

2 synthesis and characterization were moxifloxacin.

3 Q.   Dr. Zhanel, the priority application for the '942

4 patent was filed in the United States on June 30th, 1989.

5 Do you know in fact whether moxifloxacin had been

6 synthesized by that date?

7 A.   It had not.

8   MR. BERL: Let's take a look at PTX-262.

9 BY MR. BERL:

10 Q.   What is this document, Dr. Zhanel?

11 A.   This is a compound card from Bayer when Bayer

12 synthesized a compound. And in this case, this is

13 moxifloxacin, the chemical structure as shown. The date of

14 synthesis was provided on the card, and this is October the

15 4th, 1990.

16 Q.   And before October the 4th, 1990, could moxifloxacin

17 have been tested?

18 A.   No, it couldn't have been tested because it had not

19 been synthesized. You have to have the compound in order to

20 do testing.

21   MR. BERL: Let's go back to the '942 patent, Dr.

22 Zhanel, and, in particular, Column 56 of the '942 patent

23 which lists various compositions.

24 BY MR. BERL:

25 Q.   First of all, what compounds is this list of

1   compositions directed to?

2   A.   It talks about the active compounds which represents

3   the billions of compounds within the '942 patent.

4   Q.   Is this list of compositions directed particularly to

5   moxifloxacin?

6   A.   No, this is focused on all the different potential

7   billions of compounds and they're called active compounds.

8   Q.   Would a person of ordinary skill in the art

9   understand the '942 patent disclosing all of those billions

10   of compounds can be used in each of these formulations?

11   A.   No, they would not.

12   Q.   Would the person of ordinary skill in the art

13   understand Column 56 to indicate that any particular

14   compound can be used with each of the listed formulations?

15   A.   No, they wouldn't take from this that any particular

16   compound and certainly not all of the compounds could

17   potentially be used with all of these different formulation.

18   Q.   What would the person of ordinary skill in the art

19   understand this passage to mean?

20   A.   This is a laundry list. This is a laundry list of

21   any potential formulation that you can think of. And a

22   person of ordinary skill would take away from this that

23   potentially a compound can be used for a very specific type

24   of formulation if it has suitable properties.

25   Q.   Dr. Zhanel, if someone did read this passage in

1 Column 56 to disclose every combination of an active

2 compound in a listed formulation, how many such combinations

3 would there be?

4 A.   Again, it would be billions.

5 Q.   Is there any disclosure in the '942 patent of a

6 particular compound in connection with a particular

7 formulation?

8 A.   Yes, there is.

9   MR. BERL: Let's take a look at Column 53 of the

10 '942 patent.

11 BY MR. BERL:

12 Q.   What is disclosed here at the top of Column 53?

13 A.   This is a tablet formulation of Example 1 which is

14 one of the compounds that was synthesized and also one of

15 the compounds for which there are data in Table 1, the

16 microbiology data.

17 Q.   And to be clear, is the compound in Example 1

18 moxifloxacin?

19 A.   No.

20 Q.   Is there any disclosure in the '942 patent of a

21 moxifloxacin formulation?

22 A.   No.

23 Q.   Is there any disclosure in the '942 patent of any

24 ophthalmic formulation?

25 A.   No.

1 moxifloxacin relative to ciprofloxacin, some didn't look

2 that good on the resistance prevention side.

3 Q.   Were you aware of any studies relating to development

4 of resistance against pseudomonas?

5 A.   Yes.

6 Q.   And what was that information at the time?

7 A.   As of September 1998, there was data that

8 moxifloxacin was not different in terms of resistance

9 selection against Pseudomonas aeruginosa on single dose

10 exposure and there was overwhelming data that,

11 microbiologically, moxifloxacin was inferior, significantly

12 less active than ciprofloxacin than Pseudomonas aeruginosa.

13 And that is why moxifloxacin was being developed for

14 non-pseudomonal community-acquired respiratory tract

15 infections. And that was clear in September of 1998.

16 Q.   Given that moxifloxacin and ofloxacin were seen to be

17 roughly equivalent in terms of their activity against

18 pseudomonas, if moxifloxacin had been shown to be less prone

19 to the development of resistance against pseudomonas than

20 ciprofloxacin, would the advance in resistance compensate

21 for the lesser activity in terms of whether it would be

22 suitable for an ophthalmic?

23 A.   No. The number one parameter is kill the bug, dead.

24 And it was clear that moxi was significantly inferior to

25 ciprofloxacin in that regard. And that is why the art was

1   teaching away from pseudomonal infections. It says anywhere

2   you think pseudomonas is an issue, don't go there. And that

3   is why all the conclusions that were available in September

4   '98 were talking about non-pseudomonal infections such as

5   community-acquired respiratory tract infections. That was

6   clear.

7   Q.   And you think that people concerned with the

8   treatment of ophthalmic infections find that activity

9   against pseudomonas is critical?

10   A.   Yes.

11   Q.   Shouldn't be used for ophthalmic infections if it

12   doesn't treat pseudomonas?

13   A.   No, not for serious intraocular infections. For

14   conjunctivitis, you don't need pseudomonas activity but

15   that is not what the problem is that was outlined in the

16   '830 patent. You are talking about important key ocular

17   pathogens and the key ocular pathogen and the key

18   infectious diseases of the eye were intraocular

19   infections -- infections of the deep tissues of the eye.

20   And those pathogens are pseudomonas and Staph. aureus and it

21   was clear that moxi was inferior to cipro, which was the

22   market leader.

23   Q.   So treatment of deep, serious intraocular conditions

24   is what this patent is directed to?

25   A.   There is no question about that.

1   guideline, the national guideline that tells us which to
2   dissolve what drugs in, how best to dissolve them, do you
3   need co-solvents, do you need to heat, how best to get it
4   into solution. And once they are fully soluble -- and they
5   are -- there is no difference.
6   Q.   Do you understand that Bayer had internal controls on
7   their MIC testing?
8   A.   Of course.
9   Q.   So that these controls were instituted so that Bayer
10   could make comparison between the test results for compounds
11   that were done on different dates; correct?
12   A.   No, you need to be more specific. Which control are
13   you talking about? The positive controls, the negative
14   controls or the internal antibiotic controls?
15   Q.   The positive controls.
16   A.   They would have had an ATCC strain control -- and
17   they did -- to show that their technique was working. They
18   also had a sterility control but they also had internal
19   ciprofloxacin was a control. Later on, 3018 became a
20   control. And all those controls were used to see if there
21   were differences between different quinolones that Bayer was
22   synthesizing.
23   Q.   In 1998, at the time Alcon filed this patent, were
24   you aware of any information concerning the activity of
25   moxifloxacin in promoting resistance in Pseudomonas

1   aeruginosa as opposed to ciprofloxacin?

2   A.   Yes.

3   Q.   What is your understanding of what was known?

4   A.   My understanding is that two things were known and

5   well described. The first is that on repeated experiment-

6   ation, consistently in a variety of different laboratories,

7   moxifloxacin was significantly less active than

8   ciprofloxacin against Pseudomonas aeruginosa. But on

9   limited experimentation, the data showed that in terms of

10 resistance selection, using a single high inoculum study,

11 there was no difference between ciprofloxacin and

12 moxifloxacin. That is my understanding.

13   From these data, the Bayer scientists in the

14 medical community took from this that moxi is not a

15 pseudomonas drug and they started to pursue it in

16 non-pseudomonal indications such as community-acquired

17 respiratory tract infections.

18 Q.   You are discussing activity or promotion of

19 resistance?

20 A.   Yes.

21 Q.   Which?

22 A.   Both.

23   MR. HEFNER: I'd like to turn your attention to

24 PTX-1124, please.

25   1124.

BAYER HEALTHCARE AG, ALCON, INC., and ALCON MANUFACTURING, LTD.,	)))	
Plaintiffs,	))	
v.	))	Civil Action No. 06-234 (SLR)
TEVA PHARMACEUTICALS USA, INC.,	))	**HIGHLY CONFIDENTIAL -- OUTSIDE ATTORNEYS' EYES**
Defendant.	)))	**ONLY**

RESPONSIVE EXPERT REPORT OF ASHIM K. MITRA, Ph.D.

I.   Background

1.   I am the Curators' Professor of Pharmacy and Chairman of the Division of Pharmaceutical Sciences at the University of Missouri in Kansas City. I am also Vice Provost for Interdisciplinary Research at the University of Missouri, and Director for Translational Research at the University of Missouri School of Medicine. Prior to joining the University of Missouri in 1994 as a full Professor, I was an Associate Professor of Physical Pharmacy at Purdue, and before that, an Assistant Professor at Purdue and the University of Nebraska Medical Center.

2.   My expertise is in the area of drug delivery and disposition, and in particular, ocular drug delivery and disposition. I received a Ph.D. degree from University of Kansas in 1983 in Pharmaceutical Chemistry. My thesis was entitled "Passive and facilitated transport of pilocarpine across the corneal membrane of the rabbit." I have over 25 years of experience in ocular penetration, drug delivery, and disposition. My CV is attached as Exhibit 1A.

3.   As Director for Translational Research at the School of Medicine, I am involved in selecting new technologies from bench research, particularly in the ophthalmic area, and

coordinating preclinical studies for the purpose of filing an investigational new drug application with the U.S. Food and Drug Administration. As Chairman of the Division of Pharmaceutical Sciences, my responsibilities include hiring new faculty members, evaluating their performances on a yearly basis, recommending faculty for tenure and promotion, supervising all the graduate students and the staff in the division, reporting to the dean and serving on the executive committee of the School of Pharmacy.

4.   I have authored and co-authored over 200 refereed articles, published in, among other journals, International Journal of Pharmacology, International Journal of Pharmaceutics, Investigative Ophthalmology & Visual Science, Molecular Vision, Current Eye Research, The Journal of Ocular Pharmacology and Therapeutics, Pharmaceutical Research, European Journal of Pharmacology, Journal of Pharmacy and Pharmacology, Journal of Pharmaceutical Sciences, Current Drug Metabolism, Molecular Pharmaceutics, Journal of Controlled Release, Life Sciences, Expert Opinion on Drug Delivery, AAPS Pharm Sci Tech Journal, Drug Delivery, American Journal of Therapeutics, Molecular and Cellular Biochemistry, Letters in Drug Design and Discovery, European Journal of Pharmaceutics and Biopharmaceutics, Clinical Research in Regulatory Affairs and Drug Development, and Industrial Pharmacy. My work has also been presented at several universities, pharmaceutical companies and scientific organizations worldwide, the most notable being the International Conference on Ocular Diseases and Their Treatment (Germany 1997), XVI International Congress of Eye Research Conference (Australia 2004), BioMedical Transporters 2005 Conference (Switzerland 2005), Ophthalmic Drug Development and Delivery Summit (San Diego 2007), China International Drug Delivery Systems Summit 2005 (China 2005), and the Annual Meetings of the Association for Research in Vision and Ophthalmology, and the American Association of Pharmaceutical Scientists.

2

5.   I have served as Editor of the 1993 and 2003 editions of the well-known text book, Ophthalmic Drug Delivery Systems. I have authored or co-authored over twenty chapters for various texts relating to ocular drug delivery. Some of these chapters describe, among other things, the effects of physicochemical properties of drug substances and their permeation across various corneal tissues and their pharmacokinetics in ocular fluids and tissues.

6.   I serve on the editorial advisory board for Bentham Science Publishers, International Journal of Pharmaceutics, AAPA Pharm Sci, Clinical Research and Regulatory Affairs, Current Eye Research, Current Pharmaceutical Design, and Current Drug Metabolism. I also been elected to several offices and/or served as a member of various committees of the American Association of Pharmaceutical Scientists, Association for Research in Vision and Ophthalmology, and the Controlled Release Society.

7.   I actively teach numerous subjects across the pharmaceutical sciences, including drug absorption, drug transport, bioavailability and bioequivalence, and drug delivery. I have, at times, taught all of the aforementioned topics with a focus on ocular drugs. I have advised over seventy graduate students, post-doctoral fellows, resident physicians, and visiting professors and scholars.

8.   Over the course of my career, I have received numerous awards and honors, including the 2007 ARVO/Pfizer Ophthalmics Translational Research Award, the University of Missouri Curators' Professor of Pharmacy Award, the American Association of Pharmaceutical Scientists Fellow Award, the UKC Trustees Faculty Research Award, and National Collegiate Inventor of the Year Award.

9.   I am an inventor of two U.S. patents in the field of drug delivery, with one patent focused on ocular drug delivery.

10.   My recent research activities have focused primarily on three areas (1) Ocular Disposition, Metabolism and Delivery of Antiviral Agents, (2) Nasal and Pulmonary Delivery of Macromolecules, i.e., Proteins and Antisense Oligonucleotides, and (3) Oral Absorption of Novel anti-HIV Agents.

11.   I have provided expert testimony in the last four years in the following cases:

- *Allergan v. Apotex* (Ketorolac)

- *Aventis v. Apotex* (Diltiazem)

- *Novartis v. Apotex* (Calcitonin)

- *Novartis v. Apotex* (Cyclosporin)

- *GlaxoSmithkline v. Pharmascience* (Val-acyclovir).

12.   I have been retained by Williams & Connolly LLP as an expert in this litigation. I am being compensated at a rate of $500 per hour. My compensation does not depend on the outcome of this litigation. If asked, I will be prepared to present a basic tutorial to explain the pharmaceutical terms and concepts used in my expert report, and anticipated during the trial. That tutorial may include demonstrative exhibits and models. In addition to the opinions and bases set forth in this report, my testimony may include responses to facts, arguments, allegations, or references raised by Teva or its experts relating to this litigation.

13.   I have relied on the materials cited in this report, as well as my training and experience, in forming my opinions.

II.   Person of Ordinary Skill in the Art

14.   I have been asked to provide an opinion as to the qualifications of the person of ordinary skill in the art to whom the invention disclosed and claimed in Alcon's U.S. Patent No. 6,716,830 ("the '830 patent", Ex. 1B) is directed, as of September 30, 1998. Among other

things, the person of ordinary skill in the art to whom the '830 patent is directed would have knowledge regarding the treatment and prevention of ophthalmic bacterial infections by topical administration. While there are several aspects to this knowledge, as it pertains to my area of expertise and my opinions in this case, a person of ordinary skill in the art would be familiar with principles of ocular pharmacokinetics and delivery. I understand that other experts will address other aspects of the background of a person of ordinary skill.

III. Opinions

A. Summary of Opinions

15.   I have reviewed the relevant literature publicly available as of September 30, 1998 (the "priority date")[1] related to the ocular pharmacokinetics of fluoroquinolones, the physiochemical properties of moxifloxacin and other fluoroquinolones, and the other factors impacting ocular pharmacokinetics, *e.g.*, tear-protein binding, carrier-mediated absorption, carrier-mediated efflux, and melanin-binding. Based on my review of the prior art, it is my opinion that one of ordinary skill in the art would not have expected the topical ophthalmic formulation of moxifloxacin recited in claim 1 of the '830 patent (the "claimed moxifloxacin formulation") to exhibit ocular pharmacokinetic properties far superior to the ophthalmic formulations of ofloxacin or ciprofloxacin available as of September 30, 1998.

16.   Rather, if anything, the prior art would have taught a person of ordinary skill in the art that the claimed moxifloxacin formulation would exhibit ocular pharmacokinetic properties which are about the same as, or slightly inferior to, a topical ophthalmic formulation

---

[1]   I have been told by Alcon's attorneys that Teva does not dispute that the '830 patent is entitled to a priority date of September 30, 1998. I also understand from Alcon's attorneys that properties of the claimed moxifloxacin formulation discovered or reported after September 30, 1998 are relevant to the obviousness inquiry.

of ofloxacin, and which are, at most, only better than the properties of a topical ophthalmic formulation of ciprofloxacin to the extent that the prior art formulation of ofloxacin was better.

17.   I have reviewed published and unpublished data related to the ocular pharmacokinetics of the claimed moxifloxacin formulation in comparison to topical ophthalmic formulations of ofloxacin and ciprofloxacin. In my opinion, the claimed moxifloxacin formulation exhibits several beneficial properties that are far superior to those of the topical ophthalmic formulations of ofloxacin and ciprofloxacin which a person of ordinary skill in the art would not have expected as of September 30, 1998.

18.   The claimed moxifloxacin formulation has superior ocular pharmacokinetics than the topical ophthalmic formulations of ofloxacin and ciprofloxacin that were commercially available as of September 30, 1998. The moxifloxacin topically applied in the claimed formulation has these properties across the entire range of moxifloxacin concentrations (0.1% to 1.0%) recited in claim 1 of the '830 patent.

B.   Ocular Pharmacokinetics

19.   I have been instructed to assume that the reader has a familiarity with the anatomical structure of the eye given the background provided in the report of Dr. Eduardo C. Alfonso. *See* Report of Dr. Eduardo C. Alfonso ¶¶ 26-29. I agree with Dr. Alfonso's discussion of the ocular anatomy and incorporate it herein. A person of ordinary skill in the art would have been familiar with the anatomy of the eye as described in Dr. Alfonso's report.

20.   The ocular pharmacokinetics of a formulation are, in part, a characterization of the extent to which the active compound (*i.e.*, the active ingredient) penetrates the corneal layers, accumulates, and, importantly, remains at the various sites in the eye where infections form and spread. A number of factors impact the ocular pharmacokinetics of a formulation, including (a)

precorneal factors like tear-protein binding to the active ingredient; (b) the extent of passive transport through the corneal layers (which, in turn, depends on at least the lipophilicity, hydrophilicity, and molecular weight of the active ingredient), (c) any active absorption of the active ingredient through the cornea (such as by a carrier system), and (d) the rate of diffusion and efflux out of the relevant ocular tissues and away from these sites of action (which in turn depends on a variety of factors, including lipophilicity, hydrophilicity, and melanin binding within the iris and ciliary bodies).

21.   Prior to studying its pharmacokinetics, a person of ordinary skill in the art would not have known which factors will be significant for a particular formulation and how the factors will interact with one another and impact a formulation's ocular pharmacokinetics.

22.   As of September 30, 1998, as far as I am aware, there were no reported studies on the ocular pharmacokinetics of any topical ophthalmic formulation of moxifloxacin.

23.   Considering what was known at the time about how the factors mentioned above affect the pharmacokinetics of other fluoroquinolones, a person of ordinary skill in the art would not have formed a reasonable expectation that a topical ophthalmic formulation of moxifloxacin would penetrate into and achieve higher concentrations in ocular tissues far better than previously available topical ophthalmic formulations of ofloxacin and ciprofloxacin.

24.   In the absence of the only information that could have provided a reasonable expectation as to a formulation's ocular pharmacokinetic properties—actual pharmacokinetic data relating to the concentrations of the active compound in relevant ocular tissues over time—a person of ordinary skill in the art might look to other information to hypothesize about what the pharmacokinetic properties of the particular formulation possibly could be. Needless to say, this exercise is fraught with uncertainty, due to the facts that a formulation's pharmacokinetic

properties depend on a variety of factors that are not themselves predictable and that these factors interact in unpredictable ways. In order to achieve the goal of attaining high tissue concentrations of active compound in (among other relevant tissues) the cornea and aqueous humor, a compound must travel to the corneal epithelium before being eliminated by tear drainage, penetrate the cornea (passively or actively), avoid being actively effluxed back across the corneal membrane or out of the cornea or aqueous humor, avoid passive transport out of these tissues, avoid being metabolized in those tissues, and avoid being eliminated completely when the aqueous humor fluid is replenished every few hours.

25. Even after a formulation's pharmacokinetic properties are tested and understood, it is difficult (if not impossible) to ascertain which among the numerous factors affecting ocular pharmacokinetics predominate, how the factors counteract each other or otherwise interact to produce the observed result, or to what extent the various physical, chemical, or biological properties of the active compound itself impact the observed pharmacokinetic properties. Understanding the causes of a particular formulation's ocular pharmacokinetics requires an understanding of how each of the factors discussed above, which themselves are not well understood or predictable, interact in a very complicated, multifaceted system.

26. Though several of these factors, and their correlation with various physicochemical properties of the active ingredient (to the extent they are understood), are discussed below, it is important to understand that a topical ophthalmic formulation's pharmacokinetic properties are not simply a sum of the contribution of each factor, but rather the result of a complex and multi-faceted biological system artfully designed to prevent foreign compounds from penetrating into ocular tissues, and to remove those compounds that do penetrate, as quickly as possible. If a topical ophthalmic formulation's ocular pharmacokinetic

properties reliably could be predicted on the basis of simple physicochemical properties of the active compound, researchers in the field would simply design compounds that penetrate into and remain in the desired ocular tissues in high concentrations upon topical ophthalmic administration, instead of spending enormous resources testing compounds that often exhibit undesirable pharmacokinetic properties.

Precorneal Factor

27.   **Tear-Protein Binding.** Upon administration of a topical ophthalmic formulation, before an active compound reaches the corneal epithelium and has a chance to be absorbed through the corneal membrane, it can bind to tear-proteins. This tear-protein binding can unpredictably alter the tear-film concentrations of the active compound.

28.   The eye's lacrimal fluid contains a total protein content of approximately 0.7%, which consists of proteins such as albumin and globulin. Ex. 2 at 62-63 (Vincent H.L. Lee, "Precorneal, Corneal, and Postcorneal Factors" *in* Ophthalmic Drug Delivery Systems (Ashim K. Mitra ed. 1993)). The albumin content in the lacrimal fluid is about 0.4%. Ex. 3 at 10 (Indra K. Reddy & Madurai G. Ganesan, "Ocular Therapeutics and Drug Delivery: An Overview" *in* Ocular Therapeutics and Drug Delivery (Indra K. Reddy ed. 1996)). Moreover, protein content increases substantially in certain pathological conditions that affect the eye. Ex. 2 at 63 (Lee). The more tear-protein binding to active ingredient that occurs, the lower the tear fluid concentration of unbound active ingredient and therefore the less drug available for corneal penetration.

29.   As of September 30, 1998, it was known that different fluoroquinolones bind to relevant proteins at differing levels. The literature indicated that the more lipophilic the fluoroquinolone, the more protein binding occurred. Ex. 4 at 1421 (Weiguo Liu *et al.*,

Pharmacokinetics of Sparfloxacin in the Serum and Vitreous Humor of Rabbits:

Physicochemical Properties That Regulate Penetration of Quinolone Antimicrobials, Anti.

Agents Chem., 42(6):1417-1423 (1998)) (showing that ciprofloxacin (least lipophilic) binds

rabbit sera protein 23%, ofloxacin (moderately lipophilic) binds rabbit sera protein 33%, and

sparfloxacin (more lipophilic) binds rabbit sera 42%). From this data, a person of ordinary skill

in the art would have expected that the more lipophilic moxifloxacin was, the more it would bind

to tear protein, thereby leaving less free moxifloxacin available for corneal penetration.

Corneal Factors

30.   One factor that impacts the ocular pharmacokinetic profile of a topical

formulation is passive transport. Transcellular passive transport through the corneal layers is a

mechanism of ocular absorption of topical ophthalmic formulations. The rate and degree of

passive transport through the corneal layers is dependent upon many factors, including but not

limited to the lipophilicity, hydrophilicity, and molecular weight of the active ingredient.

31.   **Lipophilicity and Hydrophilicity.** The cornea is comprised mainly of three

layers: the epithelium, the stroma, and the endothelium. The corneal epithelium and

endothelium are both lipophilic (fat loving), while the middle stroma layer is hydrophilic (water

loving). Hence, a molecule needs to be lipophilic enough to pass through the epithelium and

endothelium, and yet be hydrophilic enough to pass through the stroma.

32.   As a result of this paradox, generally speaking, up to a certain point, the more

lipophilic a molecule, the more it will passively transport through the cornea layers. After

reaching that point, however, making a molecule more lipophilic (and hence less hydrophilic)

will decrease penetration. *E.g.*, Ex. 5 at 4 (Patrick M. Hughes & Ashim K. Mitra, "Overview of

Ocular Drug Delivery and Iatrogenic Ocular Cytopathologies" *in* Ophthalmic Drug Delivery

Systems (Ashim K. Mitra ed. 1993) ("Maximizing bioavailability of ophthalmic medications . . . requires the active compound be neither extremely hydrophilic or lipophilic."). The optimum value depends on each given compound or class of compounds.

33.   One indicator of the relative lipophilicity/hydrophilicity of a molecule is its octanol/water partition coefficient. The octanol/water partition coefficient can be measured by suspending the molecule in question in a flask containing equal portions of buffered water (a hydrophilic medium) and octanol (a hydrophobic medium) and assessing whether (and to what extent) a compound prefers a hydrophilic or hydrophobic environment. The pH of the buffer is important, especially for compounds such as fluoroquinolones that have different charges at different pH levels. That is because the charge of a molecule often affects the partition coefficient and, more importantly, penetration across the corneal membrane. Once a molecule partitions between the octanol and water, the concentration of the molecule is measured. The result is often expressed as a log of the ratio of the concentration of the compound in octanol to the concentration of the compound in water.

34.   All else equal, partition coefficients can be loosely correlated with the degree of passive transport across the cornea. As one might expect based on the principle discussed above that an optimal penetration requires a molecule that is neither too lipophilic nor too hydrophilic, many have reported a "parabolic" relationship (when graphed, the relationship takes the form of an inverted parabola or bell curve) between partition coefficient and penetration through the epithelium of the cornea. *E.g.*, Ex. 6 at 8 (Neil L. Burstein, "Basic Science of Ocular Pharmacology" *in Clinical Ocular Pharmacology* (2d ed. 1989); Ex. 5 at 4 (Hughes & Mitra);

Ex. 2 at 68 (Lee).[2] Though this relationship is generally understood, small changes in lipophilicity measured on a log scale would not be expected by a person of ordinary skill in the art to cause a significant change in corneal penetration. For example, in one of my studies, the log P values of Butyryl IDU and IsoButyryl IDU were calculated to be 0.875 and 0.840, and the corresponding corneal permeability values were found to be 5.36×10^{-6} cm/sec and 5.00×10^{-6} cm/sec, for Butyryl IDU and IsoButyrl IDU, respectively. Ex. 7 at 735 (Milind M. Narurkar & Ashim K. Mitra, *Synthesis, Physicochemical Properties and Cytotoxicity Studies of a Series of Novel 5'-Ester prodrugs of 5-Iodo-2'-Deoxyuridine*, Pharm. Research, 5(11):734-737 (1988)); Ex. 8 at 889 (Milind M. Narurkar & Ashim K. Mitra, *Prodrugs of 5-Iodo-2'-Deoxyuridine for Enhanced Ocular Transport,* Pharm. Research, 6(10):888-892 (1989)).

35. Though the inverted parabolic relationship between partition coefficient and penetration has been reported, optimal partition coefficient varies depending on the drug class and nature of the compounds studied. *See, e.g.*, Ex. 5 at 4 (Hughes & Mitra) (reporting that log of optimal partition coefficients are in the range of about 1 to 3 depending on the drug class); Ex. 2 at 11 (Reddy) (reporting that log of optimal partition coefficients are in the range of about 1 to 2 depending on the drug class)).[3]

---

[2]   Others have reported a sigmoidal relationship. *E.g.*, Ex. 2 at 68 (Lee, *Ophthalmic Drug Delivery*)

[3]   For example, the optimal log partition coefficient for a homologous series of n-alkyl-p aminobenzoate esters was reported to be 2.5-2.6. Ex. 9 at 241-42 (G.L. Mosher & T.J. Mikkelson, *Permeability of the n-alkyl p-aminobenzoate esters across the isolated corneal membrane of the rabbit*, Int'l J. Pharm., 2:239-43 (1979)) (temperature and pH unknown). The optimal log partition coefficient for a group of 11 steroids was reported to be 2.5-3.0. Ex. 10 at 788 (Ronald D. Schoenwald & Richard W. Ward, *Relationship between Steroid Permeability across Excised Rabbit Cornea and Octanol–Water Partition Coefficients*, J. Pharm. Sci 67(6):786-788 (1978)) (measured by at 37°, pH unknown). The optimal partition coefficient for ß-blocking agents was reported to be 2.88. Ex. 11 at 1271 (Ronald D. Schoenwald and Hong-Shian Huang, *Corneal Penetration Behavior of ß-Blocking Agents I: Physiochemical Factors*, J. Pharm. Sci., 72(11):1266-1272 (1983)) (measured at 35°, pH 7.4).

36.   As far as I am aware, as of September 30, 1998, there was no known optimal partition coefficient reported for the fluoroquinolone class.

37.   To my knowledge, the only partition coefficient data for moxifloxacin reported as of the Alcon priority date were found in a poster presented by Bayer scientists at the ICAAC meeting in 1996. Ex. 12 (U. Peterson *et al.*, *Synthesis and In Vitro Activity of Bay 12-8039, a New 8-Methoxyquinolone* (hereinafter, "Bayer Poster"). The Bayer Poster reported that in water at 25°C, the log octanol-water partition coefficient for moxifloxacin was -1.9, and in 0.1N HCl (buffered to pH 7), the log octanol-water distribution coefficient was -0.6. The Bayer Poster did not report the partition coefficients for any other fluoroquinolone. This partition coefficient data on moxifloxacin does not itself permit an evaluation of the lipophilicity and hydrophilicity of moxifloxacin relative to other fluoroquinolones. Nor does it alone provide a basis for any meaningful assessment of the compound's ability to passively penetrate the cornea.

38.   I have reviewed the literature relied upon in the expert report of Dr. Loyd Allen, as well as the other relevant literature mentioned above available as of the Alcon priority date, and I could find no study, as of September 30, 1998, which reported having measured the partition coefficient of moxifloxacin at the same time as the partition coefficients of other fluoroquinolones. However, as of the priority date, there were several studies reporting partition coefficients for other fluoroquinolones, including ofloxacin and ciprofloxacin.[4]   The table below

---

[4]   *See, e.g.*, Ex. 13A at 93 (D.B. Jack, *Recent Advances in Pharmaceutical Chemistry. The 4-Quinolone Antibiotics*, J. Clin. Hosp. Pharm, 11:75-93 (1986)); Ex. 13B at 535-36 (Keiji Hirai *et al.*, *Differences in Susceptibility to Quinolones of Outer Membrane Mutants of Salmonella typhimurium and Escherichia coli*, 29(3):535-538 (1986)); Ex. 14 at 439-40 (John S. Chapman & Nafsika H. Georgopapadakou, *Routes of Quinolone Permeation in Escherichia coli*, Anti Agents Chem. 32(4):438-42 (1988)); Ex. 15 at 2563-65 (Noriyuki Nakanishi *et al.*, *Mechanisms of Clinical Resistance to Fluoroquinolones in Staphylococcus aureus*, Anti. Agents Chem. 35(12): 2562-67 (1991)); Ex. 16 at 381-83 (Danna L. Ross *et al.*, *Physicochemical properties of the fluoroquinolone antimicrobials. III 1-Octanol/water partition coefficients and their*

shows the reported partition coefficients (converted to a log scale) for ofloxacin and

ciprofloxacin as measured in nine different studies.

	Jack pH 7.4 37°C (unknown)	Hirai pH 7.2, 25°C (aqueous phase only)	Chapman pH 7.2 unknown°C (aqueous phase only)	Nakanishi pH 7.0, unknown°C (aqueous phase only)	Ross pH 7.0, 25°C (both phases)	Takács-Novák pH 7.4 Room Temp. (aqueous phase only)	Fakuda pH 7.4, 25°C (aqueous phase only)	Montero pH 7.46 25°C (both phases)	Liu pH 7.2 25°C (both phases)
REPORTED PARTITION COEFFICIENTS FOR OFLOXACIN AND CIPROFLOXACIN									
Ofloxacin	< -2.0	-0.48	-0.71	-0.60	-0.35	-0.44	-0.64	--	-0.48
Ciprofloxacin	-1.15	-1.7	-0.82	-1.22	-0.99	-1.11	-1.22	-1.13	-\.25

39. Upon reviewing this data, a person of ordinary skill could have averaged the

reported partition coefficients for ofloxacin and ciprofloxacin, and compared the average

reported partition coefficients for ofloxacin and ciprofloxacin to the partition coefficient for

moxifloxacin reported in the Bayer Poster.[5,6] *See, e.g.*, Ex. 20 at 508-10 (Richard A. Zabinski *et*

---

relationships to structure, Int'l J. Pharm., 88:379-89 (1992)); Ex. 18 at 94 (Krisztina Takács-Novák et al., *Lipophilicity of antibacterial fluoroquinolones,* Int'l J. Pharm., 79:89-96 (1992)); Ex. 17 at Tr.1, 3-4 (Masamichi Fakuda *et al.*, *Attempts to obtain basic information concerning intraocular pharmacokinetics of fluoroquinolone antibiotics through in vitro ocular experiments*, J. Oph. Soc. Jap. 99(5):532-36 (1995)) (translation); Ex. 19 at 114 (M.T. Montero *et al.*, *Influence of physicochemical properties of fluoroquinolones on encapsulation efficiency in liposomes*, Int'l J. Pharm., 138:113-20 (1996)); Ex. 4 at 1418, 1421 (Liu).

[5] Before averaging, the person of ordinary skill in the art would have eliminated data points that appeared inaccurate. The log partition coefficient reported by Jack *et al.* of -2.0 is an outlier and is significantly different from each of the other seven data points for ofloxacin which all fall between -0.35 and -0.71. As a result, a person of ordinary skill in the art would not have included that data point in computing the average ofloxacin value. Indeed, Zabinksi *et al.* reported average log partition coefficients for ofloxacin of -0.47 based on fewer data points, which did not include the partition coefficient reported by Jack. Ex. 20 at 509-10 (Zabinski). Even if the ofloxacin data point from Jack *et al.* was included, however, the average partition coefficients of ofloxacin would be about -0.72, which is not appreciably different from the averages used herein.

[6] Because Ross *et al.* measured both the aqueous and octanol phases when measuring the partition coefficient for each fluoroquinolone, and all of Ross *et al.*'s partition coefficient determinations were made in triplicate, if a person of ordinary skill in the art were to choose one study to compare with the partition coefficient value in the Bayer Poster to assess the relative

14

al., Effect of Aerobic and Anaerobic Environments on Antistaphyloccal Activities of Five Fluoroquinolones, Anti. Agents Chem., 39(2):507-12 (1995)) (reporting average partition coefficient values for fluoroquinolones measured between pH 7.0 and 7.4). If a person of ordinary skill in the art averaged the partition coefficients presented in the table above after removing outlier data, such a person would have obtained an average reported log partition coefficient for ofloxacin of -0.52 and an average reported log partition coefficient of ciprofloxacin of -1.17. If the skilled person then compared the partition coefficient of moxifloxacin reported in the Bayer Poster (-0.6) to the average partition coefficients of ofloxacin and ciprofloxacin reported in the literature, he or she would have concluded that moxifloxacin was about as, or even slightly less, lipophilic than ofloxacin, and more lipophilic than ciprofloxacin.

40.   **Molecular Weight.** As noted, molecular weight of the drug can also play a role in the extent of passive transport. Generally speaking, the higher the molecular weight, the lower the penetration. Ex. 21 at 630 (Kaisa Mari Hämäläinen *et al.*, Characterization of Paracellular and Aqueous Penetration Routes in Cornea, Conjunctiva, and Sclera, Invest. Oph. & Visual Science 38(3):627-634 (1997)) (reporting that corneal permeability of PEGs decreased with increasing molecular weights)); *see also* Ex. 22 (Eric J. Lien & P.H. Wang, *Lipophilicity, Molecular Weight, and Drug Action: Reexamination of Parabolic and Bilinear Models,* J. Pharm. Sci., 69(6):648-650 (1980)) (reporting molecular weight having an impact on permeability); Ex. 23 (David S. Hull *et al., Permeability of the isolated rabbit cornea to corticosteroids,* Invest. Oph. 13(6):457-58 (1974) (showing that high molecular weight molecules are impeded by epithelium).

---

lipophilicity of moxifloxacin, that study would be Ross because the data in Ross appear to be most reliable.

41.   The molecular weight of a compound can be calculated from a compound's empirical formula. The molecular weights of the quinolones discussed in this report are as follows in order of increasing weight: norfloxacin = 319.3 (free base); ciprofloxacin = 331.3 (free base); lomefloxacin = 351.4 (free base); ofloxacin = 361.4 (free base); levofloxacin = 361.4 (free base); and moxifloxacin = 401.4 (free base). Thus, moxifloxacin has the highest molecular weight of any of the relevant fluoroquinolones, including ofloxacin and ciprofloxacin. Thus, all things being equal (which they never actually are), one of ordinary skill in the art would expect that moxifloxacin would not be moving faster through the intact corneal epithelium relative to other fluoroquinolones.

42.   In summary, passive transport (penetration across the cornea) is just one facet of ocular pharmacokinetics. But even with respect to this facet, it is clear that the multiple properties can have an impact. Indeed, at least lipophilicity can impact passive diffusion in multiple, contradictory ways. For instance, while up to a certain point, increasing lipophilicity correlates with increasing penetration across the corneal epithelium, the more lipophilic the molecule, the more tear-protein binding there may be, and hence the less passive diffusion of free compound across the corneal membrane. Lipophilicity and its affect on passive diffusion is simply one piece of the complex, and dynamic pharmacokinetic picture that is anything but clear and predictable.

Carrier Mediated Absorption

43.   In addition to passive transport, drugs can be transported into the cornea and into other relevant ocular tissues with the help of membrane transporter proteins or carriers. *See, e.g.*, Ex. 24 at 73 (Claude Giasson & Joseph A. Bonanno, *Facilitated Transport of Lactate by Rabbit Corneal Endothelium*, Exp. Eye Res. 59:73-81 (1994)) (demonstrating how active transport plays

a role in transport of lactate across the cornea endothelium); Ex. 25 at 20 (Johan Stjernschantz &

Maria Astin, "Anatomy and Physiology of the Eye. Physiological Aspects of Ocular Drug

Delivery *in Biopharmaceutics of Ocular Drug Delivery* (Peter Edman ed. 1993)) (demonstrating

the presence of a carrier mediated transport system in the ciliary epithelium); Ex. 26 at 815-818

(Richard A. Stone, *The transport of para-aminohippuric acid by the ciliary body and by the iris

of the primate eye*, Invest. Oph. Vis. Sci. 18(8):807-18 (1979)) (demonstrating active transport of

para-aminohippuric acid in primate iris and ciliary body). The presence of such a carrier system

can dramatically impact the ocular penetration of a molecule. When a carrier system is involved

in corneal transport, passive transport may be less important to overall corneal penetration.

44. As of September 30, 1998, a person of ordinary skill in the art would have

understood that carrier-mediated transport was a known factor impacting the ability of a drug to

be absorbed across the corneal membrane, and further would have believed that fluoroquinolones

would be transported across the corneal membrane with the help of carriers. Indeed, the

presence of a transporter on the cornea which can facilitate absorption of fluoroquinolones was

reported by Kawazu *et al.* as early as March 1998 at the 118th Annual Meeting of the "Japan

Pharmaceutical Conference" in Kyoto, Japan. *E.g.,* Ex. 27 (Goichi Kawazu *et al.*, Abstract,

118th Meeting of Pharmaceutical Society of Japan (March/April 1998) (translation).[7] An

Abstract from that meeting reported that levofloxacin transport across the entire cornea exhibited

concentration dependency in which the apparent permeability slowly reached a fixed value at a

concentration of 25mM. In addition, it was reported that levofloxacin uptake by cultured rabbit

---

[7]   *See also* Ex. 28 (Kouichi Kawazu *et al.*, *Characterization of the Carrier-mediated
Transport of Levofloxacin, a Fluoroquinolone Antimicrobial Agent, in Rabbit Cornea,* J. Pharm.
Pharmacol., 51:797-801 (1999)); Ex. 29 (Kouichi Kawazu *et al.*, *Cultured Rabbit Corneal
Epithelium Elicits Levofloxacin Absorption and Secretion,* J. Pharm. Pharmacol., 51:791-96
(1999)).

corneal epithelial cells (RCEC) reached a maximum in about 30 minutes and thereafter gradually declined. Further, in the uptake process, a concentration dependency was observed. Ex. 27 (Kawazu). Both the transport and uptake results reported by Kawazu strongly suggest the involvement of an active transport system in levofloxacin absorption through the cornea. Because fluoroquinolones were generally known to be transported across biological membranes with the help of carriers, and Kawazu reported the presence of carrier transport in the cornea, a person of ordinary skill in the art would have believed that a carrier-mediated transport mechanism would contribute to the absorption of other fluoroquinolones across the cornea. Thus, a person of ordinary skill in the art would have expected that corneal penetration of any given fluoroquinolone would likely not depend only on passive transport. Moreover, a person of ordinary skill in the art would expect that moxifloxacin, which had different chemical properties than other fluoroquinolones, would likely be actively transported into the cornea to a different degree than other fluoroquinolones but would not know whether active transport would lead to an increase or decrease in overall penetration relative to other fluoroquinolones.

Active Transport and Diffusion Out

45. **Active Transport Out.** Some molecules can be actively removed, such as by organic anionic transporters or efflux pumps, from inside a membrane to outside a membrane. Thus, even if a drug penetrates well into a membrane, if it is effluxed, the penetration is effectively counteracted and becomes far less physiologically relevant.

46. It has been known for decades that some drugs are actively transported out of eye tissues, which causes diminished concentrations in those tissues. *See, e.g.,* Ex. 30 at 723 (Michael Barza *et al., The effects of infection and probenecid on the transport of carbenicillin from the rabbit vitreous humor,* Invest. Oph. Vis. Science 22:(6):720-26 (1982)) (explaining that

B-lactam antibiotics are actively transported out of rabbit eyes via a pump in retina); Ex. 31 at 1605 (Michael Barza *et al.*, *Pharmacokinetics of Intravitreal Carbenicillin, Cefazolin, and Gentamicin in Rhesus Monkeys*, Invest. Oph. Vis. Science 24(12):1602-06 (1983)) (explaining that B-lactam antibiotics are actively transported out of monkey eyes via a pump in retina); Ex. 32 at 461 (Bernard Becker & Max Forbes, *Iodopyracet (Diodrast) transport by the rabbit eye*, Am. J. Physio. 200(3):461-64 (1961)) (reporting active transport of iodopyracet out of the eye); Ex. 33A at 485 (J.G. Cunha-Vaz & D.M. Maurice, *The active transport of fluorescein by the retinal vessels and the retina*, J. Physiol. 191:467-86 (1967)) (reporting the active transport of fluoroscein out of the retina).

47.   Drugs that are substrates for active transport systems are actively transported out of ocular tissues to different degrees, which can have a dramatic effect on drug half-life relative to other drugs in the same class. Ex. 31 at 1605 (Barza, *Rhesus Monkeys*). For example, the half-life of the B-lactam antiobiotic, Carbenicillin, in rhesus monkeys increased from 10 to 20 hours in the vitreous humor, and from 8 hours to 39 hours in the aqueous humor, upon administration of probenecid, which blocks the action of an active transporter. *Id.* In addition, the half-life of the B-lactam antiobiotic, Cefazolin, in rhesus monkeys increased from 7 hours to 30 hours in the vitreous humor, and from 7 hours to 31 hours in the aqueous humor, upon administration of probenecid. *Id.*

48.   As of September 30, 1998, it was known that different fluoroquinolones were being actively transported out of various ocular tissues at different rates. For example, Liu *et al.* reported that fluoroquinolones were being actively transported out of the vitreous, and that this efflux occurred at different rates depending on the fluoroquinolone and whether the tissue was inflamed. Ex. 4 at 1417, 1420-22 (Liu) ("We have shown that quinolones, like beta-lactam

antiobiotics, are exported from the vitreous humor via a pump which is blocked by probenecid and inflammation."). Furthermore, Liu *et al.* reported that probenecid significantly increased the half-lives of ciprofloxacin, fleroxacin, and sparfloxacin, but not ofloxacin. *Id.* at 1420-22. This indicates that ofloxacin was not as susceptible to active transport out of the vitreous as the other fluoroquinolones. *Id.* Thus, Liu demonstrated that the extent to which a fluoroquinolone is actively effluxed is not simply a function of its lipophilicity.

49.   One of ordinary skill in the art would have expected that this same type of active transport mechanism was present in the aqueous humor. As early as 1979, Reddy *et al.* reported the existence of the same active transport mechanisms in both the vitreous and aqueous humor. Ex. 33B (Venkat N. Reddy, *Dynamics of transport systems in the eye*, Invest. Oph. Vis. Sci.; 18(10):1000-18 (1979)).

50.   Furthermore, Saha *et al.* had reported the existence of a p-Glycoprotein drug efflux pump in the conjuctiva which works to restrict the overall absorption of drugs. Ex. 34A at 1221 (Pratik Saha *et. al.*, *Existence of a p-Glycoprotein Drug Efflux Pump in Cultured Rabbit Conjuctival Epithelial Cells*, Invest. Oph. Vis. Sci. 39(7):1221-26 (1998)). Because it was known to a person of ordinary skill in the art as of the priority date that the cornea is an extension of the conjunctiva and the two tissues are connected, a person of ordinary skill in the art would have expected that a p-Glycoprotein drug efflux pump existed in the cornea, which would restrict the overall absorption of drugs into the cornea.[8]

51.   Based on the available literature, a person of ordinary skill in the art would have expected that active transporters would likely play an important role in the ocular

---

[8]   This has since been proven by Kawazu *et al.* Ex. 34B (Kouichi Kawazu *et al.*, *Characterization of Cyclosporin A Transport in Cultured Rabbit Corneal Epithelial Cells: P-Glycoprotein Transport Activity and Binding to Cyclophilin*, Invest. Oph. and Visual Science, 40(8):1738-1744 (1999)).

pharmacokinetics of fluoroquinolones, but would not have known or reasonably expected how this factor would impact moxifloxacin relative to other fluoroquinolones.

52.   **Diffusion Out.** In addition to active efflux out of the ocular tissues, active compounds are also known to passively diffuse from those tissues. Like active transport out, passive diffusion out of tissues also can have a dramatic effect on the maximum concentration and half-life of a compound in the relevant ocular tissues.

53.   Liu *et al.* reported that the more lipophilic a molecule, the shorter its half-life in the vitreous humor. Ex. 4 at 4120 (Liu). Thus, sparfloxacin, one of the more lipophilic fluoroquinolones, has a shorter half-life in the vitreous than ciprofloxacin, ofloxacin, and fleroxacin. *Id.*

54.   The principle that passive transport out of ocular tissues depends, in part, on the lipophilicity of the molecule applies with equal force to passive transport out of the aqueous humor and the iris-ciliary body. Thus, for instance, others reported before the priority date of the '830 patent that the more lipophilic the molecule, the shorter the elimination half life in the aqueous and iris-ciliary body. *E.g.,* Ex. 35 (Thomas F. Freddo *et al., The source of Proteins in the Aqueous Humor of the Normal Rabbit,* Invest Oph. Vis Sci. 31(1):125-37 (1990)).

55.   From these reports, a person of ordinary skill in the art would have expected that if moxifloxacin were more lipophilic relative to other fluoroquinolones, it would have a shorter elimination half life in the various tissues of the eye. This is significant because a compound needs to stay in a tissue long enough to kill the bacteria present there. Hence, even if a person of ordinary skill in the art believed that lipophilicity would lead to higher penetration, he or she would be equally concerned that the same physiochemical characteristic would cause shorter half lives in the relevant ocular tissues.

56.   **Melanin Binding.** The iris and ciliary bodies are known to have melanin that binds to drugs and helps retain the drugs in those tissues. Because of this binding, the iris-ciliary body serves as a depot for a compound which helps to replenish the loss of drug through aqueous humor turnover, thereby extending the residence time in ocular tissues. Ex. 25 at 19-20 (Edman) (explaining that many drugs with amine groups and that are cations bind melanin in the iris, and that drug melanin complex forms a slow release system)); Ex. 6 at 10 (Burnstein) ("[T]he iris can serve as a depot or reservoir for some drugs, concentrating and then releasing them for longer than otherwise expected."); *see also* Ex. 36 at 209 (Patrick M. Hughes *et al.*, *Vitreous disposition of Two Acycloguanosine Antivirals in the Albino and Pigmented Rabbit Models: A Novel Ocular Microdialysis Technique*, J. Ocul. Pharm. Ther. 12(2):209-24 (1996)). The extent of melanin binding is thought to be dependent on the lipophilicity of the molecule. Ex. 6 at 10 (Burnstein). Because a person of ordinary skill in the art would have reasonably expected that the partition coefficient for moxifloxacin was similar to ofloxacin, he or she would have also expected to exhibit similar binding properties to melanin as ofloxacin.

C.   Ocular Pharmacokinetics of the Claimed Moxifloxacin Formulation

57.   The penetration rate into and concentration achieved in the ocular tissues is very high and unexpectedly far superior to ofloxacin and ciprofloxacin when applied in the formulation recited in claim of the '830 patent.

58.   In one study, a 0.5% moxifloxacin topical ophthalmic formulation was compared to a 0.3% ofloxacin topical ophthalmic formulation and maximal concentrations were measured in the aqueous humor, cornea, and vitreous. In the aqueous humor, maximal concentrations were 1.42 µg/ml for moxifloxacin and 0.405 µg/ml for ofloxacin, which is more than a three-fold difference. In the cornea, maximal concentrations were 24.8 µg/g for moxifloxacin and 8.01 µg/g for ofloxacin, which is about a three-fold difference. And in the vitreous, the maximal

concentrations for moxifloxacin were 0.082 µg/g and 0.003 µg/g for ofloxacin, which is about a twenty-five fold difference. Ex. 37 (Robertson *et al.*, ARVO Abstract 4906 (2004)).

59.   If one of ordinary skill in the art were to have estimated the lipophilicity of moxifloxacin relative to ofloxacin and ciprofloxacin based on the average partition coefficient values available in the prior art, and then estimated penetration based solely on partition coefficient (which, as explained above, would have been improper because it ignores other factors, *e.g.*, active transport), such a person would have estimated that moxifloxacin would reach about the same concentration as ofloxacin if applied in the same amounts topically to the eye. Taking into account the difference in the amount of moxifloxacin (0.5%) used in the study above relative to ofloxacin (0.3%), one of ordinary skill would have estimated that moxifloxacin would achieve less than a two-fold higher maximum concentration in these tissues. But as shown by this data, moxifloxacin's maximum concentration is in fact three to four times higher in the cornea and aqueous humor, and twenty-five times higher in the vitreous humor, which is entirely unexpected.

60.   Furthermore, Fukada *et al.* reported a penetration rate (permeability coefficient) for ofloxacin about 2.75 times higher than that of ciprofloxacin. Ex. 17 at 1-2 (Fakuda). Based solely on a comparison between the partition coefficient in the Bayer Poster and the average partition coefficient reported in the literature (which, again, would be ignoring other factors), one of ordinary skill would have estimated that moxifloxacin would have the same penetration rate as ofloxacin, and thus penetrate about 2.75 times better than ciprofloxacin.

61.   But this is not the case. In an Alcon study comparing the *ex vivo* corneal penetration of fluoroquinolones under steady state conditions, as shown by the graph and table attached as Ex. 38 (the table from which is reproduced below), moxifloxacin's penetration rate in

23

cm/s, *i.e.*, its permeability coefficient, is far superior to both ofloxacin and ciprofloxacin (and

much more superior to ciprofloxacin than ofloxacin is).

Ex Vivo Corneal Penetration Apparent Permeability Coefficients & Lag Time of Fluoroquinolones		
Fluoroquinolone	Permeability Coefficient (x 10^{-7} cm/sec)	Lag Time (min)
Ofloxacin	50 ± 10	66 ± 3
Ciprofloxacin	18 ± 2	70 ± 12
Norfloxacin	22 ± 2	71 ± 6
Moxifloxacin	91 ± 9	49 ± 1
Levofloxacin	29 ± 8	69 ± 13
Gatifloxacin	25 ± 2	99 ± 12
Lomefloxacin	35 ± 2	78 ± 1

62.   Indeed, this study found that moxifloxacin's permeability coefficient was nearly 2

times that of ofloxacin's permeability coefficient and five times that of ciprofloxacin's

permeability coefficient, which are unexpected results. Ex. 38 (steady state table). This study

further illustrated that at steady state, moxifloxacin concentrations in the cornea continued to

increase at a much faster (µg/min) rate than any of the other fluoroquinolones tested, and reached

a total concentration of over two times higher than ofloxacin after 300 minutes, and seven times

higher than ciprofloxacin after 300 minutes. *Id.* (steady state graph).

63.   In a separate head-to-head study between topical ophthalmic formulations of

0.5% moxifloxacin and 0.3% ciprofloxacin, Salomon *et al.* illustrated that the mean aqueous

concentration of moxifloxacin was 1.31 µg/ml, which is more than 8-times higher that the mean

aqueous concentrations of ciprofloxacin, which was 0.15 µg/ml. Ex. 39 at 468 (Renée Solomon,

Penetration of Topically Applied Gatifloxacin 0.3%, Moxifloxacin 0.5%, and Ciprofloxacin 0.3%

into the Aqueous Humor, Oph., 112(3):466-69 (2005)). Based only on the concentration

24

difference of the two formulations in the Solomon study, one skilled in the art would expect moxifloxacin's ocular tissue concentrations to be about two times (5/3) higher than that of ciprofloxacin. On the basis of the information available as of September 1998, a person of ordinary skill in the art would not have expected an 8-fold difference in the mean aqueous concentration of ciprofloxacin and moxifloxacin.

64.   This superior ocular pharmacokinetic data discussed in ¶¶ 59-64 could not have been expected on the basis of the information available in September 1998, and is not even explicable on the basis of hindsight. By way of example, the relative partition coefficients for ofloxacin and moxifloxacin gleaned from the Bayer Poster and the average reported value in the prior art simply do not explain these results.

65.   These data are also surprising given that moxifloxacin accumulates to a much greater degree than ofloxacin and other relevant fluoroquinolones in the conjunctiva, which means that there should be less moxifloxacin available to penetrate through the cornea and enter the relevant tissues via the corneal route. *See* Ex. 40 (Rudolph S. Wagner *et al.*, *Evaluation of Moxfloxacin, Ciprofloxacin, Gatifloxacin, Ofloxacin, and Levofloxacin Concentration in Human Conjuctival Tissue*, Arch. Oph., 123:1182-83 (2005)).

66.   Finally, the superior penetration of moxifloxacin is present across the entire range of moxifloxacin concentrations (0.1% to 1.0%) recited in claim 1 of the '830 patent. Data obtained using the penetration model reported by Schoenwald *et al.* shows that there is an approximately linear increase in flux (the rate at which the compound crosses the membrane) and accumulation as the concentration of moxifloxacin increases. See Ex. 41 (Corneal Perfusion Table and Graph); Ex. 11 (Schoenwald and Huang). Based on these results, the superior

pharmacokinetic properties of the moxifloxacin formulation that I have discussed in this report are present across this 0.1% to 1.0% range of concentrations.

September 19, 2007

Ashim K. Mitra, Ph.D

BAYER HEALTHCARE AG, ALCON, INC., and ALCON MANUFACTURING, LTD.,	)))	
Plaintiffs,	))	
v.	))	Civil Action No. 06-234 (SLR)
TEVA PHARMACEUTICALS USA, INC.,	))	**HIGHLY CONFIDENTIAL – OUTSIDE ATTORNEYS' EYES**
Defendant.	)))	**ONLY**

RESPONSIVE EXPERT REPORT OF GEORGE G. ZHANEL, Ph.D.

Background and Qualifications

1.   I am a Professor in the Department of Medical Microbiology, Faculty of Medicine, University of Manitoba, Winnipeg, Canada.

2.   I received a Ph.D. in Medical Microbiology from the University of Manitoba in 1994 and a Doctor in Pharmacy from the University of Minnesota in 1986.

3.   For approximately the last 20 years, the focus of my work has been antiinfectives, including quinolones (the terms "quinolone" and "fluoroquinolone" will be used interchangeably). I gave my first presentation on quinolones in 1985 while working toward my Doctor of Pharmacy. In addition, as early as 1985, I began advising clinicians regarding the use and optimal use of fluoroquinolones, and I began teaching about quinolones in 1987. The subject of my doctoral thesis (1994) in Medical Microbiology was "Cellular and Molecular Evaluation of Fluoroquinolone Resistance in *Pseudomonas aeruginosa*."

4.   I am currently the Chair of both the antibiotic (antibiotic, antimicrobial, antibacterial agent, and antiinfective will be used interchangeably) resistant infections research group and the Canadian Antimicrobial Resistance Alliance (CARA) in the Faculty of Medicine

at the University of Manitoba. I also am the Coordinator of the antibiotic resistance program in the Departments of Clinical Microbiology and Section of Infection Control, Department of Internal Medicine at the Health Sciences Centre, Winnipeg, Canada.

5. The focus of my research is the study of antibiotics in the treatment of infectious diseases. Specifically, I focus on infectious diseases caused by antibiotic resistant organisms. My antibiotic resistance research group studies antibiotic resistant infections from four main perspectives. First is epidemiology, which deals with studying the prevalence and incidence of resistant infections throughout the United States and Canada. I also study clinical epidemiology, trying to understand the patient risk factors that are associated with being infected with resistant strains of pathogens, as well as molecular epidemiology, which tries to understand what genetic types of resistant pathogens are causing infections. The second perspective is molecular diagnostics, which aims to design and test methods to rapidly, sensitively, and specifically identify resistant pathogens. The third perspective is the mechanistic ways that pathogens use to become resistant to antibiotics. Our group is actively trying to identify the cellular and molecular mechanisms of resistance that pathogens use to avoid being killed by antibiotics. Finally, the fourth and most important perspective focuses on treatment of antibiotic resistant infections. We study both existing (already marketed) and investigational antibiotics to understand their microbiological activity, molecular mechanisms of action and resistance, pharmacokinetic/pharmacodynamic properties, including Monte Carlo analyses, and influence on patient outcome from both clinical and economic perspectives.

6. These research activities, although based in Canada and focusing on resistance in Canada and the U.S., are relevant internationally to resistance issues as well. Accordingly, I have presented our research findings not only throughout North America, but also in Central

America, eastern and western Europe including Russia, Africa, the Middle East, the Far East and Australia.

7.   I currently teach medical students at the University of Manitoba in the first, second, third and fourth years of the Medical curriculum. I also teach internal medicine residents and medical microbiology/infectious diseases postdoctoral fellows. In addition, I teach Masters and Doctoral students in both Medical Microbiology and Clinical Pharmacology, as well as undergraduate students in Science, Pharmacy and Nursing. My teaching focuses on antiinfectives for the treatment of infectious diseases. My teachings cover topics such as the medicinal chemistry, mechanism of action and resistance, in-vitro activity, pharmacokinetics and pharmacodynamics, animal studies including efficacy and toxicity, and clinical uses of antiinfectives, including indications, adverse effects in humans, drug interactions and pharmacoeconomics.

8.   As evidenced from my *curriculum vitae* (attached as Exhibit 1), I have extensive experience researching, writing, presenting and teaching about antiinfectives. The focus of this work regarding antiinfectives has included mechanism of action and resistance, in-vitro activity, pharmacokinetics and pharmacodynamics, animal studies including efficacy and toxicity, and clinical uses including indications, adverse effects in humans, drug interactions and pharmacoeconomics. Although many antiinfectives have been and are being evaluated, a major focus has been on the quinolones (of which moxifloxacin is one).

9.   My work on new investigational antiinfectives has led me to serve as a consultant (through advisory boards or otherwise) to many pharmaceutical companies. These companies include Apotex, Abbott, Arpida, Astellas, AstraZeneca, Bayer, Bristol-Myers Squibb, GlaxoSmithKline, Janssen Ortho/Ortho McNeil, Kane BioTech, Leo Pharmaceuticals, Merck,

3

Micrologix, Optimer, Oryx, Pfizer/Pharmacia, Procter & Gamble, Schering-Plough, Sanofi-Aventis and Wyeth. As part of my research and consultancy role for these companies, I am routinely called on to provide advice concerning the appropriate indications for antiinfectives based on a molecule's properties, as well as to make comparative evaluations among antiinfectives. Specific topics include comparative mechanism of action and resistance, in-vitro activity, pharmacokinetics and pharmacodynamics, animal studies including efficacy and toxicity, and clinical uses including indications, adverse effects in humans, drug interactions and pharmacoeconomics. This guidance to companies on scientific matters pertains to particular indications and uses for antiinfective compounds.

10.   As part of my consulting activities, I also am called upon to assist in the identification and selection of lead compounds. This process involves, among other things, making evidence-based, comparative evaluations among antiinfectives based on their properties, providing recommendations as to which compounds should be used as reference standards for comparison, and which properties need to be optimized.

11.   I am frequently consulted by clinical specialists and generalists on matters pertaining to the treatment of infections, especially antibiotic resistant infections. These consultations focus around the selection of specific antiinfective therapy, dosage, and dosage regimen and monitoring for efficacy and toxicity, and range from accompanying physicians to bedside visits to more commonly phone calls (local or from all over North America), hallway discussions and discussions at medical/scientific meetings.

12.   From 1990-2005, I was a consultant to the Manitoba Drug Standards and Therapeutics Committee (MDSTC), a Manitoba Government committee. I was the antiinfective expert on this committee which, after assessing a new or existing antiinfective, makes

4

recommendations to the Provincial government (Manitoba Health) regarding for which indications the government should provide insurance benefits to the citizens of the Province. I was responsible for creating the guidelines for the use of quinolones (including ciprofloxacin, gatifloxacin, levofloxacin, moxifloxacin, norfloxacin and ofloxacin) in Manitoba. I have also been consulted on similar matters by other provinces and jurisdictions in Canada including Ontario Drug Benefits (ODB) and National Insured Health Benefits (NIHB).

Mandate

13.   I will testify as an expert in the area of antiinfectives, including the pharmacokinetics, pharmacodynamics, mechanisms of action and resistance, in-vitro activity, in-vivo potency, clinical efficacy, drug interactions, clinical use, and safety of quinolones (both animal toxicity and adverse effects in humans) and related antiinfectives. This will include comparing the overall properties, efficacy and suitability of moxifloxacin to other antiinfectives for treatment and/or prevention of various infections, including community acquired respiratory tract infections and important ocular infections such as keratitis and endophthalmitis. This will also include discussions of the optimal pharmacokinetic/pharmacodynamic parameters of quinolones and other antiinfectives that maximize bacterial eradication (and thus maximize clinical outcome) and minimize the development of resistance during therapy. This will also include a discussion of the selection of lead compounds.

14.   I have been asked by counsel for Bayer and Alcon to discuss the properties of moxifloxacin and other quinolones, both marketed and non-marketed.
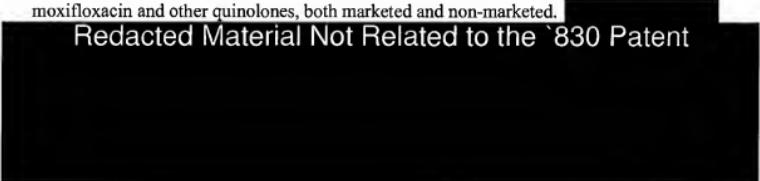
Redacted Material Not Related to the `830 Patent
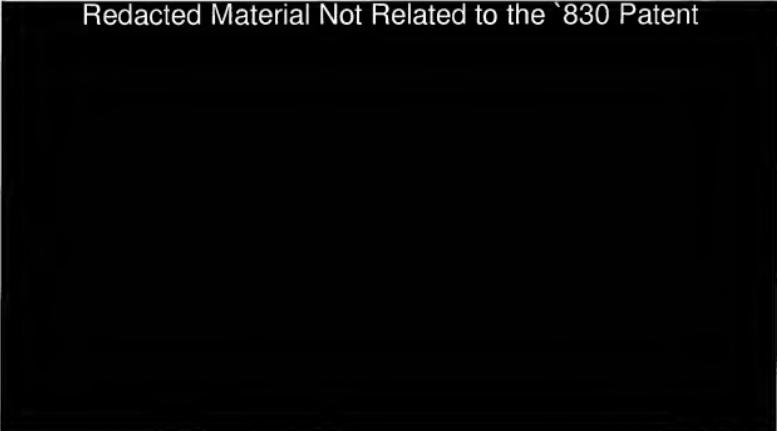
15.   In addition, I have been asked by counsel for Bayer and Alcon to discuss various

issues relating to Alcon's United States Patent No. 6,716,830 ("the '830 patent") (Ex. 6). I have

been asked to comment on the properties of moxifloxacin and other fluoroquinolones as they

would have been understood as of the September 30, 1998 priority date of the '830 patent ("the

Alcon priority date").[2] Moreover, I have been asked to discuss the factors that a person of

ordinary skill in the art would have considered in deciding whether to pursue a topical

ophthalmic formulation containing a particular antiinfective compound as of September 30,

1998, including the prevailing trends, needs, and beliefs that would have driven such a decision.

---

[2] I understand that Teva does not dispute that the '830 patent is entitled to a priority date of
September 30, 1998. Nevertheless, the opinions expressed herein would not change if a different
priority date applied to the '830 patent.

I have been asked to opine as to whether and why a person of ordinary skill in the art would have had reason to make or use a topical ophthalmic formulation containing moxifloxacin or would have been dissuaded from making or using such a formulation, on the basis of the skilled person's knowledge and available literature. In addition, I have been asked to evaluate certain properties of the claimed topical ophthalmic formulation of moxifloxacin and opine as to whether those properties would have been expected as of the September 30, 1998 priority date.

Definition of One of Ordinary Skill in the Art

16.   I understand that the person of ordinary skill in the art is a hypothetical person who may possess the combined skills of more than one actual person. I understand that because the Bayer patents and Alcon patents describe and claim different inventions, the person of ordinary skill in the art to whom they are directed may differ.
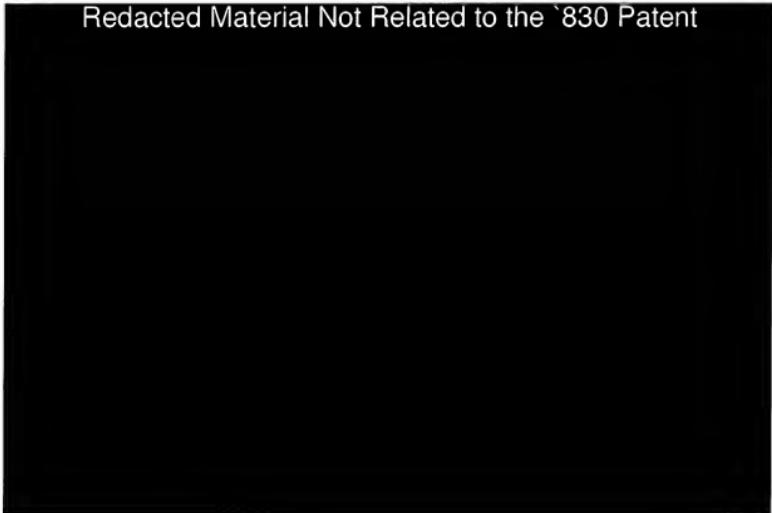
Redacted Material Not Related to the `830 Patent

18.   With respect to the issues I address in this report relating to the Alcon patent, the relevant skills and experience of a person of ordinary skill in the art to whom the Alcon patent is directed would be those of a medical microbiologist, clinician, pharmacokineticist and/or other professional having knowledge, training and experience regarding treatment and prevention of ocular infections. Such a person would have an understanding of the microbiological etiology of ocular infections, including the specific pathogens involved in various ocular infections. In addition, the person of ordinary skill would have been knowledgeable about the available and potential options for the treatment and prevention of such ocular infections as of September 30, 1998, as well as the shortcomings associated with those treatments. A person of ordinary skill in the art also would have been familiar with the manner and frequency in which topical ophthalmic antibiotic formulations were used. As such, the person of ordinary skill in the art would have been familiar with the goals of ophthalmic antiinfective therapy at the time, the needs in the field of treating and preventing ophthalmic infections, and the properties that a topical ophthalmic formulation would need to possess to meet those needs. Additionally, a person of ordinary skill in the art would have been familiar with the history and properties of antibiotics and topical ophthalmic formulations in general. Finally, a person of ordinary skill in the art would be familiar with the factors that would be considered in determining whether to make or use a topical ophthalmic formulation containing a particular antibiotic compound.

8

19.   In forming my opinions, I have relied on the materials cited throughout this report and the materials listed below, as well as my training and experience.

20.   During the last four years, I have testified as an expert at trial and by deposition in each of the following cases:

- Ortho-McNeil Pharmaceutical, Inc. v. Mylan Laboratories, Inc. (N.D. W. Va.)

- Janssen-Ortho/Ortho-McNeil Pharmaceutical, Inc. v. Novopharm, Inc. (Ontario, Canada.)

- Bayer AG, et al. v. Dr. Reddy's Laboratories, Ltd., et al. (D. Del.).

21.   I am being compensated for my time at my usual rate of $500 per hour. My compensation is in no way dependent on the outcome of this case.

22.   In addition to the specific opinions set forth in this report, I may respond to additional testimony and information that becomes available during deposition, at trial, or otherwise, including any opinions put forth by Teva's experts. I may also provide additional tables or exhibits to illustrate various aspects of my testimony. I may also refer to opinions set forth in my trial and deposition testimony from the above-referenced litigation between Bayer and Dr. Reddy, and explicitly incorporate those opinions by reference into this report.

Background Information Related to My Opinions

A.   *Pharmacokinetics/Pharmacodynamics*

23.   The combination of a drug's pharmacokinetic and pharmacodynamic properties is very important to its effectiveness in eradicating specific pathogens from specific sites of infection. That is, the drug must get to the site of the infection, in sufficient concentration such that it not only inhibits, but also kills (i.e., complete bacterial eradication) the pathogen. Bacterial killing, as opposed to mere inhibition, is very important to maximizing bacterial

9

eradication at the infectious site, clinical efficacy and minimizing the development of resistant strains.

24.   Pharmacokinetics describes the process of what the body does with the drug. This process includes: how quickly the antiinfective is absorbed and enters into the systemic circulation, the biological tissues and fluids where the antiinfective is distributed within the body, how long the antiinfective resides within the systemic circulation and how the body eliminates the antiinfective. Pharmacokinetic processes are described as ADME: A-Absorption, D-Distribution, M-Metabolism and E-Excretion. The ADME processes determine the amount of antiinfective available to interact with a particular pathogen causing the infection at a particular bodily site.

25.   Because of significant anatomical differences, the extent to which a compound reaches and remains in one particular tissue, such as the lung, or the central nervous system, does not indicate whether it will be able to reach and remain in different tissues, such as the eye. This unpredictability is even more pronounced when the method of administration of the compound changes from, for example, oral administration to topical administration.

26.   The pharmacokinetics of an antiinfective are described by various means including C_{max} (maximum concentration in plasma), t_{max} (time to maximum concentration in plasma), t ½ (half life of antiinfective in serum), AUC_{0-24} or AUC_{∞} (area under the plasma concentration-time curve over 24 hours or from time of administration to infinity) and Cl (clearance of antiinfective from the blood, which may include renal as well as non-renal clearance). Another important pharmacokinetic parameter is protein binding. Lower antiinfective protein binding is associated with greater free (non-protein bound) antiinfective being available to cross biological (human and bacterial) membranes and interact with the target

10

site leading to a biological effect. As a general matter, it is only the free fraction of an antiinfective that crosses biological membranes and exerts a biological or pharmacological effect. The symbol f is often added to symbolize the "free" fraction of an antiinfective, depicted as $f\text{AUC}_{0-24}$ or $f\text{AUC}_\infty$.

27. Pharmacodynamics describes the interaction between the antiinfective after it reaches the site of infection and the pathogen causing the infection. This interaction includes whether the antiinfective leads to a bacteriostatic or bactericidal action, whether any persistent effects will be imparted upon the pathogen after the antiinfective has been cleared from the infectious site and whether the antiinfective interacts directly or indirectly with the immune system to impart antimicrobial activity.

28. Pharmacodynamic parameters are relative to the intrinsic activity of the antiinfective in question against the specific pathogen (<u>i.e.</u>, the bug/drug interaction). This intrinsic activity of the antiinfective against a particular pathogen is designated by the MIC. As a general principle, the lower the MIC, the greater the activity of the antiinfective.

29. In pharmaceutical development, one generally wants to obtain MIC values that are as low as possible. Given that individuals working in quinolone development programs have limited time and resources, even a single dilution step (the increments for measuring MIC values), depending on the circumstances, can mean the difference between a compound that is a potential development candidate and one that is not evaluated any further. However, the person of ordinary skill would also pay close attention to the comparator compound or reference standard when considering one or two dilution-step differences.

30. The pharmacodynamics of an antiinfective are described by various means, including $f\text{AUC}_{0-24}/\text{MIC}$ (free area under the curve from 0-24 hours to the MIC), $T_{>\text{MIC}}$ (time

11

above the MIC) and C_{max}/MIC (C_{max} to MIC ratio). These pharmacodynamic parameters of an antiinfective generally correlate with its ability to eradicate the pathogen from the infectious site and lead to clinical cure. Pharmacodynamic parameters may also correlate with prevention of resistance developing during therapy. In-vitro, animal, and human studies involving fluoroquinolones have indicated that the pharmacodynamic parameter that best correlates with bacterial eradication and clinical outcome as well as prevention of resistance during therapy for quinolones is fAUC$_{0-24}$/MIC. Ex. 8 (Zhanel, J Antimicrob Chemother 2001;47:435-440); Ex. 9 (Zhanel, Curr Infect Dis Report 2001;3:29-34). Factors that affect the pharmacodynamic profile of an antiinfective include both the pharmacokinetics and the MIC of the pathogen/antiinfective pairing, meaning that the pharmacodynamic properties of an antiinfective can be altered by influencing the pharmacokinetics of the antiinfective, and/or by influencing the MIC of the pathogen/antiinfective pairing.

31.   The pharmacokinetic and pharmacodynamic data must also be viewed in the context of the purpose for which an antiinfective will be used. For example, the pharmacokinetics that describe a compound's penetration into, and retention in, the urinary tract will be important to its ability to treat urinary tract infections but far less relevant to its ability to treat an infection in the eye. Likewise, because some pathogens that are important causes of serious infections in one tissue may not cause any infection in another tissue, the pharmacodynamic data that would interest a person of ordinary skill in the art depends on the location and nature of the infections sought to be prevented and treated.

B.   *Therapeutic Index*

32.   The antimicrobial activity of fluoroquinolones is generally concentration dependent. Additionally, many unwanted adverse effects are also generally concentration dependent. The "therapeutic index," "therapeutic ratio" and "therapeutic window" are terms

12

used synonymously to describe the ratio of (1) the antiinfective concentration at which side effects occur to (2) the antiinfective concentration needed to achieve bacteriological activity and/or clinical efficacy. The higher the therapeutic index, the safer the antiinfective is at clinically effective doses. Thus an antiinfective that has higher antimicrobial activity without a commensurate increase in adverse effects or an agent that has the same antimicrobial activity but with concomitant less adverse effects will result in a higher therapeutic index and a safer drug.

33.   As with the pharmacokinetic and pharmacodynamic issues discussed above, the toxicity and therapeutic efficacy of a compound must be considered in the context of a compound's expected use. In that regard, a person of ordinary skill in the art considering a compound for treatment or prevention of a particular infection (such as an ophthalmic infection) must weigh the benefit associated with that particular use of the compound, which will depend on an analysis of the pharmacokinetic and pharmacodynamic data relating to that use, against the potential risks associated with the use of the compound. That risk-benefit ratio must then be compared to other potential therapeutic options.

C.   *Broad Spectrum Antiinfectives*

34.   While antiinfectives are frequently used as part of targeted therapy, there is a great need for antiinfectives with broad spectrum of activity because the majority of infections are treated empirically (i.e., using a particular antiinfective to treat an infection caused by an unidentified pathogen). A broad spectrum antibiotic should be bacteriologically and clinically effective against a wide variety of gram positive and gram negative bacteria, including both wild-type and resistant strains, anaerobic bacteria, and atypical pathogens such as *Mycoplasma* spp., *Legionella* spp., and *Chlamydophila* spp. In fact, the majority of seriously ill patients with infections require treatment before the identity of the pathogen can be ascertained, and broad spectrum antiinfectives can provide effective and possibly life saving empiric coverage in such

13

instances. This broad spectrum coverage will either provide suitable therapy or will provide sufficient treatment to allow the time to identify the pathogen and institute specific targeted therapy.

35.   The general principle that most infections are treated empirically holds true for the treatment and prevention of ophthalmic infections. Ex. 10 (Bower, Am. Journal Ophthalmol. 1996;121(6):712-15); Ex. 67 (Forster, CLAO Journal 1998;24(3):175-80)). In the treatment and prevention of potentially sight-threatening infections such as keratitis and endophthalmitis, an empirically used antibiotic must have a spectrum of activity sufficient to cover (either to eradicate or prevent) all of virulent pathogens that cause those infections, including *Pseudomonas aeruginosa*.

D.   *Bactericidal Activity*

36.   Rapid and effective eradication of the causative pathogen is thought to be important in minimizing patient morbidity and mortality, as well as in minimizing the development of antibiotic resistant strains. It is important to realize that the majority of both outpatient and inpatient infections are treated empirically based on the knowledge of the most likely causative pathogens. An agent that has powerful bactericidal activity against the implicated pathogen needs to be administered as quickly as possible to maximize patient outcome. Selecting the correct antiinfective the first time around – that is, the one that is active and bactericidal against the infecting pathogen – is paramount to optimal patient outcome and minimizing the development of antibiotic resistant organisms. I like to remind clinicians that "dead bugs don't mutate," which means that the best way to minimize organisms from mutating and becoming resistant to antiinfectives is to kill them dead. Dead organisms will not become antiinfective resistant pathogens.

14

E. *Streptococcus pneumoniae*

37.   Since the 1980s, North America and the rest of the world have witnessed a rapidly growing incidence of pathogens concomitantly resistant to one or more classes of antiinfectives. Pathogens resistant to antibiotics cause a variety of infectious diseases ranging from bacteremia to urinary tract infections to skin soft tissue infections. However, infections of the respiratory tract – one of the most common kinds of infections in the world – have been most affected by the worldwide spread of resistant pathogens. Respiratory infections may also be associated with systemic manifestations such as bacteremia and meningitis. Major respiratory pathogens that have developed resistance to antibiotics worldwide include *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*. Of these respiratory pathogens, the one causing greatest concern is *S. pneumoniae*: it is by far the most important clinically in terms of breadth of infections caused, it has a significant percentage of strains that are resistant to one or more classes of antiinfectives, it commonly afflicts patients, and it causes significant morbidity and mortality. Ex. 12 (Doern, Antimicrob. Agents Chemother., 2001;45:1721-1729). *S. pneumoniae* also is the major bacterial cause of community acquired respiratory infections such as community acquired pneumonia (CAP), acute exacerbated chronic bronchitis (AECB) and acute sinusitis.

38.   *S. pneumoniae* are increasingly becoming resistant to antiinfective classes such as penicillins, cephalosporins, macrolides, tetracyclines, sulfonamides – and even quinolones – and also are becoming multidrug resistant (MDR) pathogens. Ex. 13 (Chen, N. Engl. J. Med., 1999;341:233-239. Serious morbidity and mortality have resulted in patients with respiratory infections infected with antibiotic resistant strains of *S. pneumoniae*. As *S. pneumoniae* is the number one bacterial cause of respiratory tract infections such as pneumonia and bronchitis, and as resistance to commonly used antibiotics continues to escalate worldwide, there is concern that

these MDR pathogens may be or become untreatable, resulting in increased patient morbidity and mortality. As such, there is a need for antibiotics to treat *S. pneumoniae*, including for treatment of MDR strains.

39.   Both ciprofloxacin and ofloxacin possess sub-optimal antibacterial activity and poor pharmacodynamic activity against *S. pneumoniae*. This has resulted in clinical failures in seriously ill patients with community acquired pneumonia, limiting their use for treatment of such respiratory infections.

40.   Levofloxacin demonstrated that it was bactericidal versus wild-type fluoroquinolone-susceptible *S. pneumoniae* (fAUC$_{0-24}$/MIC $\geq$ 35). This lead to its rapid adoption by the medical community for the treatment of both outpatient and inpatient community acquired pneumonia. However, levofloxacin is not active against first-step (ParC mutants) fluoroquinolone-resistant *S. pneumoniae*. These fluoroquinolone-resistant *S. pneumoniae* have been reported by Chen and colleagues in the New England Journal of Medicine in 1999, and now are commonplace globally. Ex. 13 (Chen). More worrisome have been reports, such as Davidson et al. in the New England Journal of Medicine in 2002, of clinical failures using levofloxacin in patients with respiratory tract infections caused by levofloxacin resistant *S. pneumoniae* strains. Ex. 14 (Davidson, N. Engl. J. Med., 2002;346:747-750). Some of these patients have developed levofloxacin resistance while on levofloxacin therapy.

41.   The problem of organisms becoming resistant to antiinfectives surfaced within a few short years after the discovery and clinical use of penicillin in World War II. Thus, development of resistance is not new and is a continuously growing threat to classes of antiinfectives worldwide. The growing incidence of antiinfective resistance dramatically escalated in the 1980s with the rising numbers of immunocompromised patients, the continued

increase in world travel, etc. Resistance to antiinfectives is a growing global problem with many organisms and especially with penicillin-resistant and multi-drug resistant *S. pneumoniae*.

42.   In Canada, a large amount of ciprofloxacin was used for the empiric treatment of outpatient community acquired respiratory infections. Given that the major pathogen causing community acquired respiratory infections is *S. pneumoniae,* and knowing that ciprofloxacin achieves poor pharmacodynamic activity against this pathogen, it was not surprising that Canada documented a very high incidence of ciprofloxacin-resistant *S. pneumoniae*. The microbiological community has understood from the report published by Chen and colleagues in the New England Journal of Medicine in 1999 that if one uses antiinfectives that do not kill the pathogen (e.g., *S. pneumoniae*) it may promote resistance development. Ex. 13.

43.   To further complicate things, it is critical that we have existing antibiotics (like moxifloxacin) that not only have activity against resistant and MDR pathogens, but also are bactericidal against these pathogens and minimize the development of resistance development on therapy.

F.   *Mycobacterium tuberculosis and Non-Tuberculosis Mycobacterium avium Complex*

44.   Tuberculosis caused by *Mycobacterium tuberculosis* remains one of the deadliest diseases in the world. The World Health Organization (WHO) estimates that more than eight million new cases of tuberculosis occur each year and approximately 2-3 million people die from the disease each year. In addition, it is estimated that approximately one in six people globally are infected with *M. tuberculosis*. The major problems with current treatments include rapidly escalating antibiotic resistance to commonly used agents such as isoniazid and rifampin, serious adverse effects of available agents and the extensive length of current treatment regimens (ranging from six months to more than two years).

17

45.   Other mycobacterium species such as non-tuberculosis *Mycobacterium avium* complex ("MAC") are also important causes of patient morbidity and mortality. MAC refers to a serotypical complex of two distinct species *Mycobacterium avium* and *Mycobacterium intracellulare*. As the two species are indistinguishable by available genetic methods, patients are said to be infected with MAC. MAC infections are becoming more common especially as causing pulmonary disease and disseminated MAC disease in patients with HIV/AIDS. The major problems with current treatments include rapidly escalating antibiotic resistance to commonly used agents including macrolides, serious drug interactions and adverse effects of available agents and the extensive length of current treatment regimens (ranging from one year to a lifetime).

    G.   *Ophthalmic Infections*

46.   With regard to ophthalmic infections, the most feared pathogens capable of being sight-threatening and even eye threatening when they infect the cornea or anterior chamber of the eye include *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Ex. 15 (Eifrig, Ophthalmology, 2003;110:1714-17); Ex. 16 (Alfonso, Am J. Ophthalmology, 1986;101:429-33); Ex. 17 (Goldstein, Ophthalmology, 1999;106(7):1313-18). As of the Alcon priority date, the emerging resistance of ophthalmic pathogens to current therapies – including and especially quinolone therapies such as ophthalmic ciprofloxacin – would have been a grave and growing concern for a person of ordinary skill in the art. Ex. 18 (Goldstein, Invest. Ophthal. Vis. Sci., 1998;39(4):4951-B702); Ex. 19 (Chaudhry, Arch Ophthalmol 1998;116:1251); Ex. 20 (Snyder, Am. Journal Ophthalmol. 1992;114:336-38); Ex. 21 (Knauf, Cornea 1996;15(1):66-71); Ex 22 (Hodge, Invest. Ophthal. Opt. Sci. 1995;36(4):754-662); Ex. 23 (Maffett, Am. Journal Ophthalmol. 1993;115(4):545-46); Ex. 24 (Alexandrakis, Ophthalmology, 2000;107(8): 1497-1502); Ex. 17 (Goldstein); Ex. 25 (Chaudhry, Am. Journal Ophthalmology, 1999;128(4):509-

10); Ex. 26 (Kunimoto, Ophthalmology 1999;106(1):80-85); Ex. 27 (Garg, Ophthalmology, 1999;106(7): 1319-23); Ex. 28 (Hwang, Survey Ophthalmology 2004;49(2):S79); Ex. 29 (Blondeau, Survey of Ophthalmology, 2004;49(2):S73-78). This concern was especially acute given the widespread systemic and ophthalmic use of formulations containing quinolones in the late 1990s. See, e.g., Ex. 30 (Ball, Exp Opin Invest Drugs 1998;7(5);761-783).

H. *Brief History of Fluoroquinolones*

47.   Within the antiinfective arsenal belong many different classes, including penicillins, cephalosporins, carbapenems, macrolides, sulfonamides, aminoglycosides, tetracyclines and quinolones. It is important to have many different classes of antiinfectives because the different classes: (1) provide different mechanisms of action and resistance, (2) possess different antimicrobial activity and hence different indications for use, (3) offer the potential to use combination therapy to achieve additive or synergistic activity or to broaden antimicrobial spectrum, (4) utilize different metabolic or excretory pathways and (5) may help avoid or minimize specific adverse effects including allergies.

48.   In addition, with the ever growing and inevitable development of resistance, it is important to have antiinfectives that are active against pathogens resistant to other chemical classes. That is because it was assumed, as of both the Bayer priority date and Alcon priority date, that compounds within the same class of quinolone antibacterials killed bacteria by the same mechanism, so that development of resistance to one member of the class would translate into development of resistance to another member of the class.

49.   Nalidixic acid, the first marketed quinolone (actually a naphthyridine), was synthesized in the early 1960s. Although it was introduced into clinical use in the mid-1960s, its weaknesses limited its use. These shortcomings included lack of activity against many clinically important gram-negative bacilli such as *Pseudomonas spp.*, poor inhibition of gram-positive

organisms, failure to attain adequate serum concentrations, high frequency of resistance development, and a high incidence of adverse effects. Although nalidixic acid was not itself a very useful antiinfective, it did represent a new synthetic chemical class within the antiinfective armamentarium and did possess activity against certain resistant organisms. More important, the discovery of this compound sparked the ensuing quinolone era and development of new antimicrobial agents.

50. Modification of the nalidixic acid structure led to the synthesis of the first fluoroquinolone, norfloxacin, which exhibited enhanced activity for gram-negative organisms, including *Pseudomonas aeruginosa*. However, due to its poor attainment of adequate systemic concentrations, its utility was limited to the treatment of non-systemic infections, such as infections of the urinary tract.

51. Further chemical substitutions of the fluoroquinolone molecule resulted in the development of ciprofloxacin in the 1980s, which relegated norfloxacin to an unimportant drug. Ciprofloxacin possessed a broad antimicrobial spectrum, with more potent gram-negative activity than previous compounds and somewhat improved activity against gram-positive organisms. It also portrayed favorable pharmacokinetics that allowed for twice-daily dosing for the treatment of systemic infections and a low potential for adverse effects. These factors led to ciprofloxacin becoming an industry standard for the treatment of systemic infections by July 1988. Years after it was introduced to treat systemic infections (FDA approval date October 1987), and after it had successfully and safely treated infections in millions of patients worldwide, ciprofloxacin was introduced in a topical ophthalmic formulation (trade name, Ciloxan®, FDA approval date December 1990). Ex. 31 (FDA approvals for ciprofloxacin tablets, ciprofloxacin topical ophthalmic formulation).

52.   However, because ciprofloxacin was optimized for activity against gram negative bacteria, a major goal of quinolone research in the late 1980s was to find a broader spectrum quinolone that retained the safety of ciprofloxacin and its enhanced potency against gram negative bacilli such as *Pseudomonas aeruginosa*, but demonstrated enhanced activity against gram positive cocci such as *Streptococcus pneumoniae*.

53.   Ofloxacin was introduced shortly after ciprofloxacin. Like ciprofloxacin, ofloxacin somewhat improved on the activity of prior quinolones against gram-positive organisms, but it displayed less improvement against gram-negative organisms than ciprofloxacin. It also portrayed favorable pharmacokinetics that allowed for twice-daily dosing and a relatively low potential for adverse effects (except for central nervous system effects). Years after it was demonstrated to safely and effectively treat systemic infections in millions of patients (FDA approval date December 1990), ofloxacin was introduced in a topical ophthalmic formulation (trade name, Ocuflox®, FDA approval date July 1993). Ex. 32 (FDA approvals for ofloxacin tablets, ofloxacin topical ophthalmic formulation).

54.   Levofloxacin – the S-enantiomer of ofloxacin – was developed subsequently. It possessed greater antibacterial activity than ofloxacin, while maintaining low toxicity. However, levofloxacin-resistant *S. pneumoniae* developed associated with clinical levofloxacin failures. As such, more powerful fluoroquinolones than levofloxacin against *S. pneumoniae* were needed (discussed further below).

55.   In addition to ofloxacin and levofloxacin, numerous other quinolones have been synthesized and evaluated clinically before and since the development of ciprofloxacin. However, these compounds have been almost uniformly plagued by adverse effects. Ex. 33 (Rubinstein, Chemother, 2001;47(suppl3):3-8. Quinolones are, to my knowledge, one of the

21

most toxic class of drugs ever developed. To find a quinolone compound that is safe and effective is to find the very rare exception to the general rule for fluoroquinolones that the higher the in-vitro and in-vivo potency, the greater the toxicity.

56.   Among the most troubling aspects of quinolone toxicity are the widely disparate nature of the severe adverse reactions they cause and their propensity to present only after administration to many thousands—if not millions—of patients. Moreover, it is important to keep in mind that the examples of toxicity discussed herein are nowhere near exhaustive, as numerous other quinolone compounds have proven unacceptably toxic and fell off the radar without full explanation in the scientific literature. In addition, it should be noted that each quinolone discussed below that presented unacceptable toxicity in humans demonstrated an absence of such toxicity during in vitro and in vivo (animal) testing that is a scientific, regulatory, and ethical pre-condition to initiation of human testing.[3]

57.   Quinolone toxicity started with nalidixic acid, which was the first quinolone introduced in 1962. This quinolone was plagued with adverse effects especially involving the central nervous system. These adverse effects were initially documented in the 1960's and 1970's and included serious adverse effects such as intracranial hypertension. The high incidence of adverse effects as well as the seriousness of potential adverse effects greatly limited the use of nalidixic acid. Ex. 34 (Paton, Drug Safety 1991;6(1):8-27).

58.   In the mid and late 1980s, the development of numerous promising quinolones was halted or their use severely limited following the revelation of toxicity following human use.

---

[3] In discussing the development, use, termination, and restriction of quinolone compounds in this report, I am referring to the United States market, which is most relevant to the question of whether the person of ordinary skill in the art would consider a compound acceptably non-toxic. The person of ordinary skill in the art would not have reason to pursue the use of a compound that would not be considered safe in the United States.

High doses of norfloxacin (daily dosage to 1200 mg or 1600 mg) resulted in crystalluria (precipitation of the drug in the urine). Ex. 35 (Swanson, Antimicrob Agents Chemother 1982;23:284-288). This side effect prevented increasing the daily dose of norfloxacin to produce a serum concentration high enough for the treatment of systemic infections, thus limiting treatment of localized infection such as of the urinary tract. Enoxacin demonstrated great potential as a therapeutic agent until it was associated with drug interactions (with theophylline and non-steroidal anti-inflammatory agents) resulting in severe consequences such as seizures. These drug interactions reported between 1985-1990 led to termination of the development of this agent. Exs. 33-34 (Rubinstein, Paton). Fleroxacin demonstrated excellent potential as a therapeutic agent until it was associated with phototoxicity ranging from mild to severe skin reactions. These phototoxic skin reactions were reported initially in approximately 1989 and led to termination of the development of this quinolone Ex. 33 (Rubinstein). Difloxacin also demonstrated excellent potential as a therapeutic agent until it was associated with severe central nervous system adverse effects. These central nervous system adverse effects include severe psychotic reactions reported initially in 1989 and led to termination of the development of this quinolone. Ex. 36 (Smith, Antimicrob Agents Chemother 1989;33:1721-23). Flumequine displayed promising potential as a therapeutic agent until it was associated with severe central nervous system adverse effects. These central nervous system adverse effects include convulsions initially reported in 1990 and lead to termination of the development of this quinolone. Ex. 37 (Christ. J Antimicrob Chemother 1990;26(SupplB):219-25); Ex. 38 (Defoin, J Toxicol Clin Exp 1990:10:469-472). To my knowledge, most of these compounds were never developed for use in treating ocular infections by administration of a topical ophthalmic formulation.

59.   The newer, more active quinolones known before the Alcon priority date were just as toxic, if not more so. Temafloxacin, a promising fluoroquinolone that was used extensively in various countries in the early 1990s, was withdrawn very suddenly in 1992 when, four months after FDA approval and use in thousands of patients, it was associated with haemolytic uremic syndrome, coagulopathy and renal (kidney) failure. Ex. 39 (Norrby, Drugs 41993;5(Suppl3):59-64; Ex. 33 (Rubinstein). Lomefloxacin was approved for use in humans in the United States in 1992, but was withdrawn from the market shortly after due to phototoxicity and CNS side effects, including convulsions. Ex. 33 (Rubinstein). Sparfloxacin was approved for use in humans in the United States in 1996, but was withdrawn from the market due to phototoxicity and QTc prolongation and consequent worry about cardiac arrhythmias. Ex. 33 (Rubinstein). In the first half of the 1990s, as discussed in more detail later in this report, Bayer's compound BAY Y3118 demonstrated phototoxicty in human tests and was terminated. Ex. 33 (Rubinstein).

60.   Then, in 1998-99, three very active and potentially promising compounds— tosufloxacin, trovafloxacin, and grepafloxacin—all were terminated as a result of toxicity upon use in humans, in the form of eosinophilic pneumonitis and other immune-related reactions, hepatic failure and cardiac arrhythmias, respectively. Ex. 40 (Kimura, Nihon Kokyuki Gakkai Zasshi, 1998;36:618-622 (Abstract)); Ex. 33 (Rubinstein). Around the time of the Alcon priority date, on the basis of the public literature, these were among the quinolones that were of greatest interest to practitioners in the antiinfective field. All three of these examples are instructive.

61.   Tosufloxacin was a very active and broad spectrum quinolone. Ex. 41 (Fernandes, Antimicrob Agents Chemother 1988;32:27-32). Early clinical tests did not reveal significant toxicity. Ex. 42 (Suzuki, Hinyokika Kiyo 1989;35:717-26 (Abstract)); Ex. 43 (Aoki,

Kansenshogaku Zasshi 1989;16(7)3:593-605 (Abstract)); Ex. 44 (Sakurai, Hinyokika Kiyo 1994;40(3):279-84 (Abstract)); Ex. 45 (Fukushima, Kinyokika Kiyo, 1992;38(4):501-06 (Abstract)). However, in July 1998, tosufloxacin was revealed, following clinical testing in humans, to cause eosinophilic pneumonitis (a dangerous pulmonary condition). Ex. 40 (Kimura). The compound also was revealed to cause thrombocytopenia and nephritis. Ex. 33 (Rubinstein). As a result of these toxicities, the development of the compound was terminated. Exs. 40, 33 (Kimura, Rubinstein). I am not aware of any effort to make or use a topical ophthalmic formulation containing tosufloxacin.

62.   Trovafloxacin, developed by Pfizer, demonstrated a substantial improvement of activity against gram-positive pathogens along with retention of activity against gram-negative pathogens compared to ciprofloxacin (including *Pseudomonas aeruginosa*). In addition, due to the discouraging history of quinolone toxicity, the Phase III clinical trials for trovafloxacin tablets was almost unprecedented in size—approximately seven thousand patients were enrolled. The clinical trials for trovafloxacin (as well as previous in-vitro and animal testing) revealed no toxicity, and the FDA approved trovafloxacin tablets in December 1997. See, e.g., Ex. 45 (Leophonte, Eur. J. Clin. Microbiol. Infect Dis., 1998;17(6):434-40); Ex. 46 (O'Doherty, Eur. J. Clin. Microbiol. Infect Dis., 1998;17(6):441-46); Ex. 47 (Tremolieres, Eur. J. Clin. Microbiol. Infect Dis., 1998;17(6):447-53); Ex. 48 (Williams, Eur. J. Clin. Microbiol. Infect Dis., 1998;17(6):454-58); Ex. 49 (Jones, Am. J. Med., 1998;104(1):28-32); Ex. 50 (June 9, 1999 FDA Advisory). Approximately 2 years and 2.5 million treated patients later, trovafloxacin was revealed to be associated with severe liver failure, with some patients requiring organ transplantation and others dying as a result of fulminant hepatic failure. Ex. 33 (Rubinstein); Ex. 50 (June 9, 1999 FDA Advisory). The exact mechanism of toxicity remains unclear.

Trovafloxacin was withdrawn from the market on January 6, 2000. I am unaware of any effort to develop a topical ophthalmic formulation containing trovafloxacin.

63.   Likewise, the compound grepafloxacin was very active and was approved by the FDA in November 1997 after extensive in-vitro, animal and human testing revealed no toxicity. Ex. 51 (Chodosh, Antimicrob. Agents Chemother. 1998;42(1):114-20); Ex. 52 (Hook, Antimicrob. Agents Chemother., 1997;41(8):1843-45). After approximately 2 years and over a million treated patients later, grepafloxacin was associated with QTc prolongation manifesting as cardiac arrhythmias. Ex. 33 (Rubinstein). The mechanism of the QTc prolongation was blockage of the delayed potassium rectifier channel in the heart, even at low concentrations. Grepafloxacin was withdrawn from the market in late 1999. Once again, this deadly toxicity was not revealed until exposure to over a million of patients. To my knowledge, no development of a topical ophthalmic formulation of grepafloxacin ever was pursued.

64.   The compounds clinafloxacin and gemifloxacin also were known as of the Alcon priority date. These compounds were known to be very active against both gram-negative and gram-positive pathogens. Ex. 53 (EP Application 0195316 A1); Ex. 54 (Wise, Antimicrob. Agents and Chemotherapy, 1998;42(2):428-30); Ex. 55 (Cormican, Antimicrob Agents Chemother 1997;41:204-211). However, after extensive human use, these compounds—like almost all other quinolone antibiotics—were found to be unacceptably toxic. Clinafloxacin caused severe phototoxicity, and gemifloxacin caused hypersensitivity (rashes) in approximately one-third of female patients. Ex. 33 (Rubinstein); Ex. 56 (Gemifloxacin (Factive), The Medical Letter, 2004;46(1192):78-79). I am unaware of any effort to pursue a topical ophthalmic formulation containing any of these compounds.

65.   The one quinolone compound other than moxifloxacin that was developed as a topical ophthalmic formulation after the Alcon priority date was gatifloxacin. After extensive clinical testing first reported in 1995 (Ex. 57 (Nakashima, Antimicrob Agents Chemother, 1995;39(12):2635-40) (Abstract)) and clinical use in millions of infected patients, it was introduced in a topical ophthalmic formulation (trade name, Zymar®) in 2003. After gatifloxacin was developed as a topical ophthalmic formulation and demonstrated a lack of toxicity upon such administration, systemic use of that compound was associated with enhanced risk of hyper and hypoglycemia in high risk patients (elderly, diabetic patients with renal dysfunction taking concomitant insulin and/or oral hypoglycemics). Ex. 58 (Canadian Adverse Reaction Newsletter 2003;13(3)). Gatifloxacin tablets recently were withdrawn from the market.

66.   As this non-exhaustive summary indicates, though certain toxicities (such as the cardiac arrhythmias associated with sparfloxacin and grepafloxacin) are shared by more than one member of the class, most of the toxicities are difficult to identify and predict. In other words, though the quinolone antibiotics have caused toxicity in almost every vital organ system, one cannot simply conduct a few in-vitro and animal tests and have reason to believe that toxicity will be avoided. On the contrary, these toxicities almost invariably have avoided detection in animal testing (which is why they were administered to humans) and often required extensive use in thousands or millions of infected patients to identify. However, while the exact nature of the toxicity a quinolone will cause is difficult to determine, a person familiar with this history, in the absence of data demonstrating extensive and safe human use, would predict and expect that a new quinolone would cause an unacceptable toxicity.

I.   *The Treatment and Prevention of Ophthalmic Infections as of September 1998*

67.   Both at the priority date of the Alcon patent and today, the most important ophthalmic infections that concerned persons of ordinary skill in the art are found in the cornea

and interior tissues of the eye. The ocular infections keratitis and endophthalmitis were considered important because these ocular infections were not always adequately treated and prevented with existing therapies, and because, if not prevented and/or treated properly, they were sight-threatening infections. Ex. 67 (Forster); Ex. 7 (Steiner, Am. Journal of Ophthalmology 1991;112:10S-14S).

68.   Topical ophthalmic formulations containing ofloxacin and ciprofloxacin, as well as formulations containing non-quinolone antibiotics, were used in the treatment and prevention of these ocular infections. In order to treat and prevent corneal infection (keratitis) and endophthalmitis, a compound must reach the site of infection and be present in a sufficient concentration, and/or for adequate time, to prevent or eradicate the infection. The suitability of a topical ophthalmic formulation containing a particular compound therefore depends on the particular ocular pharmacokinetics of the formulation, its pharmacodynamics (the ability to eradicate the particular pathogens that are most likely to cause the sight-threatening infections that would have been of greatest concern to skilled artisans), and the potential toxicity associated with such treatment and prevention. All of these factors must be considered and weighed against the currently available therapies, to assess the risk/benefit ratio and to determine whether a new topical ophthalmic formulation should be made and used.

69.   Among the most clinically important and virulent pathogens that caused corneal infections and endophthalmitis as of the Alcon priority date were *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Therefore, in analyzing the data regarding the activity of a compound, such as moxifloxacin, a person of ordinary skill in the art of ophthalmic infections

would focus on those pathogens (and others) that are implicated in sight-threatening eye infections, including *Pseudomonas aeruginosa*.[4]

70.   Importantly, bacteria are not static populations; rather, they are constantly mutating and evolving and becoming less susceptible and/or resistant to the therapies to which they have been exposed. This concern about growing resistance development was acute in the area of ophthalmic infections in the late 1990s, in light of escalating reports of ocular strains that were resistant to currently-available quinolone therapy. Exs. 17-29.

71.   Therefore, the goal of a person of ordinary skill in the art at the time of the Alcon priority date was to develop a topical ophthalmic formulation that could effectively treat quinolone-resistant ocular pathogens without becoming clinically obsolete as a result of resistance development. Generally speaking, resistance to antibiotics is a class problem: a strain that develops resistance to one quinolone usually will be resistant or less susceptible to another quinolone. The solution to this problem is to use therapies with different mechanisms of action, so that a mutation in a bacterial enzyme that caused resistance to, for example, a quinolone like ciprofloxacin, would not impact the susceptibility to those antimicrobials that possess a different mechanism of action. Ex. 59 (Zurenko, Expert Opin Investig Drugs. 1997;6(2):151-8). Because widespread use breeds resistance, and quinolones had been used extensively in systemic infections, ophthalmic infections, veterinary medicine, and even agriculture for many years by 1998, a person of ordinary skill in the art would not have believed that another quinolone was the preferred solution to the growing problem of quinolone resistant ophthalmic infections. Ex. 60

---

[4] It should be noted that different strains of the same pathogens found in two different tissues will be different, and therefore may have different susceptibility to a given antibiotic. For that reason, compounds are tested against strains isolated from ocular tissues in order to evaluate their potential for treating ophthalmic infections, not just systemic strains that may not be predictive of ocular pharmacodynamics.

(Ashley, Medical Laboratory Sciences, 1986;43:157-62) (associating agents' maintenance of activity against ocular pathogens with a low level of systemic use).

72.   In evaluating the suitability of a compound for use in treating and preventing ophthalmic infections, one other important factor must be considered. Topical ophthalmic formulations are usually used, both prophylactically and in treatment, empirically—that is, without knowledge of the identity of the pathogen being prevented or treated. Exs. 10; 67 (Bower; Forster). For that reason, a useful topical antibiotic formulation must have a broad spectrum of activity that covers all of the important potential ocular pathogens. A person of ordinary skill would not have been interested in a formulation that is more potent than the current therapy against some important ocular pathogens but is less potent against other important ocular pathogens. Such a formulation could potentially improve clinical efficacy in treating and preventing some ocular pathogens, while reducing clinical efficacy against other important ocular pathogens, which given the evolution of bacteria and concerns regarding developing resistance—would have been discouraging to a person of ordinary skill in the art.

J.   *The Public Record Regarding Moxifloxacin and other Fluoroquinolones as of September 1998*

73.   I have reviewed the references relied upon in the expert report of Dr. Loyd Allen relating to moxifloxacin, as well as the other literature relating to moxifloxacin that was available as of the September 30, 1998 Alcon priority date. As of that date, there was far less public data concerning moxifloxacin than many of the compounds discussed above, including trovafloxacin, tosufloxacin, and grepafloxacin. The data that had been published was quite discouraging with regard to the suitability of moxifloxacin to treat ophthalmic infections by topical administration.

74.   In particular, there was a paucity of data relating to the potential toxicity of moxifloxacin. In that regard, the only published information of which I am aware related to animal tests to evaluate certain specific toxicities—phototoxicity and seizures—and very limited administrations to a small number of young, healthy volunteers. See, e.g., Exs. 4-5 Bremm Declarations; Ex. 61 (Stass, Antimicrob Agents Chemother 1998;42(8):2060-65); Ex. 62 (Schmuck, Antimicrob Agents Chemother, 1998;42(7):1831-36). The public record did not reveal any results of human testing in patients with systemic or local infections, let alone provide information that could provide a skilled person with an expectation that an active quinolone like moxifloxacin would not be unacceptably toxic, in the form of data demonstrating that a sufficient number of patients with infections had been effectively and safely treated, when treated with routine dosing over several days.

75.   To my knowledge, the only reported human testing of moxifloxacin involved administration of moxifloxacin to a small number of healthy, young volunteers. See, e.g., Ex. 61 (Stass). Adverse events, generally, are low probability events, and are more likely to occur in patients (not healthy volunteers) who are older, frequently have co-morbid diseases and are ill from their infectious disease. Toxicity, therefore is revealed upon administration of multiple doses of a drug (as it would be used clinically) to thousands (if not millions) of sick patients who have an infection, are elderly and frequently have multiple medical problems and frequently are being administered concomitant medication. The administration of a single dose or even multiple doses to a small cohort of healthy, young volunteers is a pre-requisite to the more extensive, multiple-dose administration to sick patients with an infectious disease. Pharmacokinetic studies in a small cohort of healthy volunteers (whether administered a single dose or multiple doses) do not provide toxicity data on which a person of ordinary skill in the art

31

would rely to form a belief (or modify an existing belief) as to the lack of toxicity of a compound, especially in a class of compounds—such as quinolones—in which unacceptable toxicities repeatedly have been revealed only following extensive Phase III clinical testing in sick, infected patients and post-approval use. See, e.g., Ex. 33 (Rubinstein); Ex. 39 (Norrby).

76.   For the reasons discussed above, pre-clinical animal tests are even less predictive of quinolone toxicity upon widespread use in humans. In each of the examples discussed above, in which the use of a quinolone was terminated or severely restricted due to toxicity, the toxicity was not detected in pre-clinical animal testing.

77.   The toxicity data presented in the Bremm declarations (discussed in greater detail below) was limited to a single specific toxicity: phototoxicity.[5] Exs. 4-5. As discussed above, the skilled person's expectation that an active quinolone would be toxic does not emanate from concern about a single specific toxicity associated with the drug class. Rather, the quinolones have been associated with numerous serious and often life-threatening toxicities in a variety of organs, so that data that addresses only one specific toxicity (in this case phototoxicity)—even if it provided confidence that the particular toxicity at issue would potentially not arise in humans—would not modify the expectation that another unacceptable toxicity, whether previously associated with quinolones or not, would arise upon use in humans. For that reason, the phototoxicity data presented in the Bremm declarations, or the ex-vivo animal testing of rat

_____

[5] For that reason, as discussed below, any statement in the Bremm declarations relating to tolerability would be understood to refer to phototoxicity. To the extent that such a statement would be understood more broadly, a person of ordinary skill in the art, given the absence in the Bremm declarations of any toxicity data that does not relate to phototoxicity, would not regard it as meaningful or significant. A person of ordinary skill in the art would not interpret the Bremm declaration to disclose or suggest that moxifloxacin has been demonstrated to be safe upon widespread, multiple dose testing in infected humans (or for that matter any human testing at all), which, as discussed herein, is the only testing that potentially could modify the skilled person's expectation that moxifloxacin would be unacceptably toxic.

brains to predict seizure-induction presented in the literature (Ex. 62) (Schmuck), would not have changed the expectation that moxifloxacin would demonstrate unacceptable toxicity that would not permit use in humans.

78.   Indeed, in September 1998, following the revelation that sparfloxacin caused (among other toxicities) cardiac arrhythmias and the first reports associating tosufloxacin with eosinophilic pneumonitis, a person of ordinary skill in the art would have been especially concerned and pessimistic regarding the toxicity of quinolones whose safety had not been demonstrated by extensive human testing in infected and otherwise sick patients with a variety of infectious diseases. Other compounds such as temafloxacin, trovafloxacin and grepafloxacin that had been so tested in humans—and even approved by the FDA—likewise proved unacceptably toxic as well, in conformance with a skilled artisan's expectation. Given the absence of such extensive human testing of moxifloxacin, and the toxic history of active quinolones, a person of ordinary skill in the art, on the basis of the public record, would have expected that moxifloxacin would exhibit unacceptable toxicity.

79.   While there was little toxicity data relating to moxifloxacin published by September 1998, there was a substantial amount of in vitro activity data that had accumulated. The data were rather consistent and showed that moxifloxacin was more active than ciprofloxacin against a variety of gram-positive pathogens and generally less active than ciprofloxacin against a variety of important gram-negative pathogens. Ex. 63 (Fass, Antimicrobial Agents and Chemotherapy, 1997;41:1818-24); Ex. 64 (Woodcock, Antimicrobial Agents and Chemotherapy, 1998;41(1):101-06; Ex. 65 (Bauernfiend, J. Antimicrobial Chemotherapy 1997;40:639-51); Ex. 30 (Ball). Moxifloxacin's diminished activity compared to ciprofloxacin was especially pronounced with *Pseudomonas aeruginosa*, a critically important

gram-negative pathogen known to cause a variety of systemic and ocular infections.[6] For

example, the 1996 scientific abstract (Poster) published by Bayer on which Dr. Allen relies,

reports that moxifloxacin was approximately 8x less active than ciprofloxacin against the tested

strains of *Pseudomonas aeruginosa*. Ex. 66 (1996 Bayer Poster). The published articles cited

above, which tested more strains, reported approximately the same reduced activity of

moxifloxacin compared to ciprofloxacin against *Pseudomonas aeruginosa*.

        80.   As of the 1998 priority date, numerous quinolones had been reported in the

literature that far surpassed ciprofloxacin's activity against gram-positive pathogens—such

activity was, by that time, neither unusual nor noteworthy. The desired activity profile was a

compound that improved on the activity of ciprofloxacin against gram-positive pathogens that

are relevant to a specific use of a compound (e.g., *S. aureus*) while maintaining (or at least nearly

maintaining) ciprofloxacin's activity against important gram-negative pathogens, including

Pseudomonas aeruginosa. Compounds that fit that desired activity profile had been reported in

the literature as of the September 1998 Alcon priority date, including trovafloxacin, tosufloxacin,

sparfloxacin, grepafloxacin and clinafloxacin. Ex. 65 (Bauernfiend); Ex. 64 (Woodcock); Ex. 41

(Fernandes); Ex. 55 (Cormican); Ex. 30 (Ball). From the standpoint of in vitro activity, which

comprised nearly all of the publicly available information regarding moxifloxacin as of the

Alcon priority date, moxifloxacin appeared to be a far less attractive option than other

quinolones. This is especially true when one focuses on a particular use—the treatment and

prevention of ophthalmic infections—where *Pseudomonas aeruginosa* plays a major role.

---

[6] As discussed below, *Pseudomonas aeruginosa* would have been an especially important
pathogen to a person of ordinary skill in the art of ophthalmic infections.

81.   In addition to the information discussed above, Dr. Allen also relies on the '942 patent in support of his contention that the '830 patent is invalid. A person of ordinary skill in reading any reference, including the '942 patent, would first look to the data to determine what a reference teaches or suggests. I have analyzed the '942 patent and have found not a single piece of data relating to moxifloxacin (not even microbiological data), even though data are presented for several other compounds in columns 57 and 58. Of course, no data are presented relating to the toxicity of moxifloxacin, the pharmacokinetics of moxifloxacin (in the eye or any tissue), the bacteriological or clinical efficacy of moxifloxacin in treating systemic and/or ophthalmic infections or quinolone-resistant infections, or the ability of moxifloxacin to prevent the development of resistance.

82.   The subject of the '942 patent is a new genus of chemical quinolone compounds—called "compounds of the invention"—that are purported to be active antibacterial agents. Moxifloxacin is one compound within the genus of "compounds of the invention" that I understand includes millions of potential congeners. I also understand that moxifloxacin is one of many compounds disclosed in Table 1 of the '942 patent. The '942 patent does not describe any formulation of moxifloxacin; it does describe a tablet formulation for another compound. Ex. 3 at Col. 53. Nor does the '942 patent disclose that moxifloxacin is appropriate for any particular use (such as topical administration for ophthalmic use) or disclose at what particular dosage range moxifloxacin should be used. I therefore disagree with Dr. Allen's statement that the '942 patent discloses and anticipates a topical ophthalmic formulation containing 0.1-1% moxifloxacin, as later claimed in the '830 patent.

83.   Rather, the '942 patent discloses general uses, formulations, and concentration ranges for the compounds of the invention. No person of ordinary skill in the art would

understand the statements regarding the compounds of the invention, such as the language in column 54 of the '942 patent relating to the diseases that "can be prevented, alleviated, or cured by the compounds according to the invention," to indicate that any of the compounds—let alone each of the compounds—is useful in preventing, alleviating, or curing each of the approximately fifty listed indications. To the contrary, a person of ordinary skill in the art would understand that no compound ever synthesized has been able to do so; he would never interpret this statement, especially in the absence of any supportive data, to mean that each of these millions of compounds—or any particular compound within the genus—could do that which no compound in human history accomplished. Instead, a skilled person would understand that the compounds of the invention are antibiotics and, depending on their particular pharmacokinetic, pharmacodynamic, and toxicological properties, may be useful to treat one or more of the listed diseases.

84. Likewise, a person of ordinary skill in the art would not understand the disclosed concentration range of 0.1 to 99.5% (or the more preferred range of 0.5 to 95%) to disclose any useful information at all. It most certainly does not provide information regarding any particular concentration within that very broad range that should be used for any particular compound of the millions disclosed in any particular formulation for any particular disease. Dr. Allen's effort to read the '942 patent to describe that one particular compound of the millions disclosed (moxifloxacin) can be used in one particular concentration range within the broad concentration range of the '942 patent compound in one particular formulation among many disclosed to treat one type of infection among many disclosed is simply contrary to how a person of ordinary skill in the art would have interpreted the reference. The fact that moxifloxacin is claimed in the '942 patent does not modify this conclusion.

85.   A person of ordinary skill in the art interested in new topical ophthalmic formulations would not look to the '942 patent. It discloses no topical ophthalmic formulations and provides no information whatsoever regarding the issues relevant to treating and preventing ophthalmic infections, including microbiological activity and bacteriological and clinical efficacy against ocular infections caused by *Pseudomonas aeruginosa*, microbiological activity and bacteriological and clinical efficacy in treating quinolone-resistant ocular strains, the ability to prevent resistance development, ocular pharmacokinetics, and systemic and ocular toxicity. The '942 patent discloses none of this information—it would have been considered essentially useless for a person of ordinary skill in the art interested in a new topical ophthalmic antibiotic formulation. Rather, such a person of ordinary skill would have begun with a reference disclosing topical ophthalmic formulations, such as the formulation containing ciprofloxacin, and would have looked for ways to modify and improve the formulation. That is the closest prior art. For the reasons discussed above, on the basis of the information available in September 1998, the substitution of moxifloxacin for ciprofloxacin in a topical ophthalmic formulation would have been considered a step in the wrong direction.

K.   ***A Person of Ordinary Skill in the Art Would Not Have Had Reason to Make or Use a Topical Ophthalmic Formulation Containing Moxifloxacin***

86.   I have been asked to evaluate whether, on the basis of the information available in September 1998, a person of ordinary skill in the art would have had reason to make or use a topical ophthalmic formulation containing moxifloxacin. As discussed above, I have regularly consulted with physicians (generalists and specialists), scientists, companies and governments for the last two decades regarding the use of antibiotics in the treatment and prevention of infectious diseases, including infections of the eye. In evaluating whether a particular compound

should be used to treat or prevent disease in a particular organ, such as the eye, several factors

must be considered, including:

- The goals and needs in the field of treating and preventing ophthalmic infections;

- The currently available therapies used to treat and prevent infections in the eye;

- The purposes for which the formulation being evaluated would be used;

- The microbiological etiology of the infections that must be treated and prevented;

- The pharmacokinetic properties of a topical ophthalmic formulation containing the compound, especially in the areas of the eye where infections must be treated and prevented;

- The location within the eye of the infections that must be treated and prevented;

- The pharmacodynamic data relating to a compound's ability to eradicate the relevant pathogens (infections);

- The potential systemic and ocular toxicity associated with use of the compound to treat and prevent ophthalmic infections;

- A comparison of the potential benefit of making or using a new topical ophthalmic formulation with the risks associated with its use;

- The data and experience regarding other uses of the compound to treat infections in humans;

- The potential impact of the new formulation on resistance development to the entire antibiotic class or chemically unrelated classes;

- Alternative options that could be pursued instead, including how the factors above pertain to those alternative options.

87.   In concluding that the invention of the '830 patent was obvious, I note that Dr.

Allen does not consider most of these factors. His analysis thereby is at odds with the process

that a person of ordinary would have undertaken in considering the suitability of a particular

compound to treat ophthalmic infections by topical administration of a formulation.

38

88.   As discussed above, the state of the art therapies at the Alcon priority date for the treatment and prevention of the infections that would have concerned a person of ordinary skill included topical ophthalmic formulations containing ciprofloxacin and ofloxacin. While those formulations were effective in treating and preventing many infections, there was a need for improvement, in particular with regard to a growing proportion of ocular strains that were resistant to these two therapies. Exs. 17-29. Nevertheless, aside from ocular irritation associated with crystallization of topical ophthalmic ciprofloxacin, these therapies did not cause significant toxicity—ciprofloxacin and ofloxacin are among the safest topical and systemic fluoroquinolones ever used.

89.   The goal of a person of ordinary skill in the art at the Alcon priority date therefore would have been to develop a formulation containing a compound as safe as ciprofloxacin and ofloxacin that could treat and prevent corneal infections and endophthalmitis, including those infections caused by *Pseudomonas aeruginosa* and infections that were resistant to available quinolone therapy, and to which resistance would not quickly develop.

90.   Among the expected uses of a new topical ophthalmic formulation that would have been the focus of a skilled artisan, prophylaxis was by far the most common. Though keratitis is an important infection to treat, it is relatively rare. See Ex. 67 (Forster, CLAO Journal 1998;24(3):175-80); Ex. 11 (Masket, J. Cataract Refract Surg., 1998;24:725-26). By contrast, the surgeries requiring antibiotic prophylaxis to prevent post-operative infections, including LASIK surgery and cataract surgery, were very common as of the priority date and expected to become even more common, due to the increased popularity of the former and the aging of the population that drives the latter. See Ex. 68 (Javitt, Arch Ophthalmol. 1991;109:1085-89) (reporting that more than a million cataract surgeries are performed in the

United States per year); Ex. 69 (Donnenfeld, J. Cataract Refract Surg., 2005;31(10):2008-11); Ex. 70 (Solomon, J. Cataract Refract Surg., 2003;29(10):2001-06). The occurrence of endophthalmitis or keratitis following these surgeries, though potentially devastating, is very rare—in the range of 0.1%. Ex. 71 (Kattan, Ophthalmology, 1991;98(2):227-38); Ex. 72 (Aaberg, Ophthalmology, 1998;105:1004-10); Ex. 69 (Donnenfeld); Ex. 70 (Solomon). For this reason, the most common use for which a person of ordinary skill in the art sought a new ophthalmic formulation was to prevent already rare events of keratitis and endophthalmitis following surgery.

91. In this context, the concerns and expectations regarding toxicity are especially crucial, since a practitioner certainly does not want to cause a significant adverse event by administering an antibiotic to a patient who is neither infected nor likely to suffer from a threatening ophthalmic infection if administered the currently-available, very safe therapy. A person of ordinary skill in the art of the Alcon patent would therefore have an extremely low tolerance for toxicity risk, compared to (for example) a practitioner considering whether to make or use a formulation to treat a potentially lethal lung infection (e.g., community acquired pneumonia caused by a resistant pathogen) in a very sick, elderly patient with co-morbid illnesses.

92. Given the rarity of the infections, the predominance of prophylactic use, and the efficacy of topical ciprofloxacin, a person of ordinary skill in the art would not have reason to make or use a topical ophthalmic formulation containing a compound he expected to be toxic. As explained above, a person of ordinary skill in the art, on the basis of his knowledge and the literature available in September 1998, would have expected moxifloxacin to be toxic. What would not have been as predictable is the exact nature of the toxicity and whether it would

present upon topical ophthalmic administration. For example, the topical ophthalmic administration of the antibiotic chloramphenicol caused fatal cases of aplastic anemia. See Ex. 73 (Fraunfelder, American Journal of Ophthalmology 1982;93:356-60); Ex. 74 (Fraunfelder, Medical Toxicology 1987;2:287-93). Prolongation of the QT interval—a cardiac arrhythmia that has been associated with certain quinolones such as sparfloxacin and grepafloxacin—likewise is a toxicity that has been observed following topical ophthalmic administration of the antiglaucoma drug timolol maleate. Ex. 74 (Fraunfelder). In the absence of data demonstrating either that moxifloxacin is not unacceptably toxic or that its toxicity will not arise following topical ophthalmic administration to humans, a person of ordinary skill in the art would be dissuaded from making, using, or researching a topical ophthalmic formulation containing moxifloxacin, due to the expectation that the compound—like almost all other active quinolones—would be toxic.[7]

93.   Moreover, as discussed above, *Pseudomonas aeruginosa* is one of the most important and devastating causes of keratitis and endophthalmitis. Ex. 16 (Alfonso); Ex. 17 (Goldstein); Ex. 15 (Eifrig); Ex. 27 (Garg); Ex. 18 (Goldstein). A person of ordinary skill in the art would not have been interested in making or using a topical ophthalmic formulation that was not expected to at least maintain the efficacy of the available ciprofloxacin formulation in treating and preventing *Pseudomonas aeruginosa* infections in the cornea and interior ocular tissues.

94.   In essence, a formulation that would have been expected to have improved bacteriological and clinical efficacy against gram positive pathogens but significantly less against *Pseudomonas aeruginosa* would not have been viewed as advantageous. Even worse,

---

[7] Quite surprisingly, as discussed in greater detail below, moxifloxacin is not unacceptably toxic.

since the mutation rate for *Pseudomonas aeruginosa* is high to all antimicrobial classes, including quinolones, any new therapy made approximately eight years after the introduction of ophthalmic ciprofloxacin, would require it to be significantly more active than ciprofloxacin against this critical ocular pathogen. Just as the bacteria evolve and become more powerful, so too must the therapies. Moreover, the introduction of a compound expected to be less bacteriologically and clinically effective than ciprofloxacin would only be expected to accelerate the problem of resistance, since use of such a compound would provide an opportunity for ocular *Pseudomonas aeruginosa* strains to mutate and develop even faster resistance upon exposure to moxifloxacin, rather than be eradicated upon exposure to ciprofloxacin. As stated previously, moxifloxacin microbiologically was not as active as other fluoroquinolones, and a person of ordinary skill in the art would not have expected it to treat and prevent ophthalmic infections caused by *Pseudomonas aeruginosa* as effectively as the existing Ciloxan® therapy. Ex. 65 (Bauernfiend); Ex. 64 (Woodcock); Ex. 41 (Fernandes); Ex. 63 (Fass); Ex. 30 (Ball); Ex. 55 (Cormican); Ex. 66 (1996 Poster); Ex. 75 (Diamond, British Journal of Ophthalmology, 1995;79:606-09). As I have observed and stated many times, including above, dead bugs don't mutate. Taken together, the available data regarding moxifloxacin's reduction in *Pseudomonas aeruginosa* activity compared to ciprofloxacin and the emergence of fluoroquinolone-resistant ophthalmic infections would have dissuaded a person of ordinary skill in the art from making or using a topical ophthalmic formulation of moxifloxacin.

95. Moreover, given the etiology of the important ocular infections, and the importance of *Pseudomonas aeruginosa*, other fluoroquinolones would have been more attractive candidates for topical ophthalmic use than moxifloxacin. For example, trovafloxacin, tosufloxacin, clinafloxacin, grepafloxacin, and gemifloxacin, all displayed superior activity

against *Pseudomonas aeruginosa* compared to moxifloxacin and improved gram positive activity compared to ciprofloxacin. Exs. 65, 64, 41, 30, 55, 63; 66. To be clear, a person of ordinary skill in the art would not have pursued topical ophthalmic formulations of these compounds either, due to concerns about toxicity. However, to the extent that one ignores this crucial factor and relies principally on in vitro activity data (as Dr. Allen does, but a person of ordinary skill in the art would not have done), these other quinolones would have been more attractive candidates for a topical ophthalmic formulation than moxifloxacin.

96.   In addition, as discussed above, a person of ordinary skill in the art would have been concerned about the emerging resistance of ocular infections to quinolone therapy. To the extent that resistance to a new quinolone therapy emerged quickly, as expected, it would have provided only temporary benefits over the existing therapies, rather than a long-term solution that the person of ordinary skill in the art would have sought. The solution to this problem—finding a compound with a different mechanism of action—would have led practitioners away from pursuing a formulation containing a quinolone and towards antibiotics outside the quinolone class. Ex. 59 (Zurenko); Ex. 76 (Ysasaga, Invest. Ophthal. Vis. Sci. 2001;42(4):1352); Ex. 77 (Jones, Antibacterial Agents and Chemotherapy, 1987;31(4):625-29). It is not surprising, therefore, that microbiologists in the late 1990s were focused on finding and using compounds from new classes of antibiotics with different mechanisms of action, rather than just finding new members of classes to which resistance was developing at an alarming pace.

97.   Quite surprisingly, moxifloxacin—despite being a fluoroquinolone—does possess a different mechanism of action from ofloxacin and ciprofloxacin, by effecting bacterial eradication through dual binding (rather than principally binding to only one target) to two

43

different bacterial enzymes (DNA gyrase A and Topoisomerase IV). Ex. 28 (Hwang); Ex. 78 (Pestova, J. Antimicrob Chemother 2000;45:583-90); Ex. 79 (Ong-Tone, J. Cataract Refract Surg 2007;33:59-62). The dual binding mechanisms of action of moxifloxacin inhibiting both DNA gyrase and topoisomerase IV, limits the development of resistance of moxifloxacin compared to ciprofloxacin. Ex. 29 (Blondeau). As a result, resistance to moxifloxacin has emerged far more slowly than expected.

98. In order to treat and prevent corneal infections and prevent endophthalmitis, the topical administration of a formulation must lead to sufficient concentration of compound at the site of infection and/or for the appropriate duration of time to eradicate the bacteria. I am not aware of any data available in September 1998 regarding the ocular pharmacokinetic properties of moxifloxacin. Nor can those properties be adduced from pharmacokinetic data relating to different tissues, such as the lung or heart, upon systemic administration, due to the differences in anatomy and physiology in each tissue. I know of no way to predict or reasonably expect the ocular pharmacokinetics of a topical ophthalmic formulation of a compound based on the data that was available regarding moxifloxacin in September 1998.[8]

99. Ophthalmic moxifloxacin solution has been successful in treating and preventing corneal infections and preventing endophthalmitis, despite its diminished *Pseudomonas aeruginosa* activity, due in large part to its pharmacokinetic properties. Ophthalmic moxifloxacin solution penetrates into, accumulates, and remains in the aqueous humor and cornea at far greater concentrations than prior art quinolones. See, e.g., Ex. 80 (Kim, Current Medical Research and Opinion, 2005;2H(1):93-94; Ex. 81 (Kim, Ophthalmology,

---

[8] I understand that an expert report in this case is being submitted by Dr. Ashim Mitra. I understand that Dr. Mitra's research focuses on (among other things) ocular pharmacokinetics.

2005;112:1992-96); Ex. 82 (Solomon, Ophthalmology, 2005;112:466-69); Ex. 83 (Robertson, Invest. Ophthal. Vis. Sci., 2004;45:ARVO E-Abstract 4906); Ex. 85 (Thibodeaux, Current Eye Research 2004;28(5):337-42); Ex. 79 (Ong-Tone). A person of ordinary skill would not have expected, on the basis of the information available in September 1998, that topical ophthalmic moxifloxacin would be as effective as ciprofloxacin in treating and preventing the ophthalmic infections that were of greatest concern, including those caused by *Pseudomonas aeruginosa*—they would have expected moxifloxacin to be less effective. Yet, quite surprisingly, topical ophthalmic moxifloxacin is as effective as topical ophthalmic ciprofloxacin against this important ocular pathogen. Ex. 87 (Aliprandis, Cornea, 2005;24(2):201-205; Ex. 88 (Learning, J. Cataract Refract Surg, 2004;30:892-900); Ex. 89 (Schlech, Survey Ophthalmology, 2005;50(Supp.1)S7-15; Ex. 85 (Thibodeaux); Ex. 28 (Hwang); Ex. 29 (Blondeau); Ex. 79 (Ong-Tone).

Redacted Material Not Related to the `830 Patent

Redacted Material Not Related to the `830 Patent

Redacted Material Not Related to the `830 Patent

50

Date: September 19, 2007

_____
George G. Zhanel, Ph.D.

BAYER HEALTHCARE AG, ALCON, INC.,)
and ALCON MANUFACTURING, LTD.,)
)
            Plaintiffs,)
)
)
             v.)
)
TEVA PHARMACEUTICALS USA, INC.,)
)
            Defendant.)
)

**CONTAINS CONFIDENTIAL
INFORMATION PURSUANT TO
PROTECTIVE ORDER**

Civil Action No. 06-234 (SLR)

RESPONSIVE EXPERT REPORT OF EDUARDO C. ALFONSO, M.D.

I.   INTRODUCTION

    1.   I am a Professor and the Edward WD Norton Chair In Cornea and External

Diseases in the Department of Ophthalmology at the Bascom Palmer Eye Institute, University of

Miami, in Miami, Florida. I graduated from Yale University Medical School in 1980, after

which I was an intern at Mt. Sinai Hospital, a resident in Ophthalmology at Bascom Palmer Eye

Institute, and a fellow in Cornea and External Diseases, Ophthalmic Pathology at Harvard

Medical School and the Massachusetts Eye and Ear Infirmary. I have attached as Exhibit 1 ("Ex.

1") a curriculum vitae that describes in detail my educational background and the highlights of

my career.

    2.   My work at Bascom Palmer involves treating patients who have complicated

ophthalmic infections, including mis-diagnosed and/or post-operative bacterial infections that

can cause sight loss. In addition, I consult on difficult cases of bacterial infections. I also

perform ophthalmic surgeries, and an important component of my surgical work involves the

prevention of infection during and after surgery. I am familiar with the products that have been

used to prevent and treat ophthalmic bacterial infections over the last two decades, including products containing fluoroquinolone antibiotics, such as Ciloxan®, Zymar®, and Vigamox®. I have used each of these products to treat and prevent bacterial infections in patients.

3.   In addition to my work treating patients and performing surgery, I conduct research relating to the treatment and prevention of ophthalmic infections. I have served as the Medical Director of the Bascom Palmer Ocular Microbiology Laboratory for more than 20 years. In this capacity, I train post-doctoral research fellows in ocular microbiology. Among other topics, my research has focused on the ability of various ophthalmic formulations (both marketed and non-marketed) to treat and prevent ophthalmic bacterial infections, as well as the properties of those formulations. This research in microbiology includes the use of standard techniques used to evaluate activity (often measured by minimum inhibitory concentration, or "MIC") and efficacy of compounds and formulations against ocular pathogens (disease causing organisms). For this purpose, I maintain a library of clinical isolates of ocular pathogens that are used to evaluate potential antimicrobial treatments. The evaluation of the properties of formulations containing fluoroquinolones for ophthalmic use has been a significant area of my research. I have authored hundreds of book chapters, monographs, and peer-reviewed publications, many of which relate to ophthalmic infections and the treatment and/or prevention thereof. A list of my publications is included in my CV, exhibit 1.

4.   In connection with my microbiological research, I have consulted with various companies in obtaining and assessing data regarding topical ophthalmic antibiotic formulations, including for the purpose of advising whether to pursue (or to continue to pursue) development of a product. I serve on the advisory board for ophthalmic antibiotic development for several companies and for more than a decade have advised companies as to whether and why topical

ophthalmic antibiotic formulations (including those containing fluoroquinolones) should be made, pursued, and used.

5.   I frequently serve as a peer-reviewer for articles relating to ophthalmic infections for numerous journals and have served on several editorial boards of peer-reviewed journals, including Archives of Ophthalmology, Ocular Surgery News, and Eye World ALACCSA.

6.   For nearly two decades, I have taught ophthalmology to medical school students, residents, interns, and fellows, including instruction regarding the treatment and prevention of ophthalmic bacterial infections, as well as the properties and uses of ophthalmic formulations containing fluoroquinolones. I have served on the faculty of numerous national and international meetings relating to the diagnosis and treatment of ocular infections and the properties and uses of ophthalmic formulations containing fluoroquinolones.

7.   I have been retained as an expert witness by the law firm of Williams & Connolly LLP for this litigation. I am being paid at my usual hourly consulting fee, at a rate reflected in exhibit 2. My compensation does not depend on the outcome of this case.

8.   I have reviewed the expert report of Dr. Loyd Allen, Jr., submitted by Teva in this litigation, as well as the exhibits to that report. I have also considered the documents discussed throughout this report. I may also prepare additional exhibits to illustrate various aspects of my testimony before trial.

9.   During the last four years, I have not testified as an expert at trial or by deposition except in *Otero v. Garcia*, KPD2002-0234(806) (Puerto Rico) and another medical malpractice case in Puerto Rico.

10.   I intend to testify regarding the state of the art as known on or before September 30, 1998, which I understand is the priority date of U.S. Patent No. 6,716,830 (Ex. 3, which I will refer to as the "'830 patent" or the "Alcon patent" or the "patent in suit"), and the qualifications of a person of ordinary skill in the art at that time.

11.   In my opinion, based on the state of the art as of September 30, 1998, a person of ordinary skill would not have had an interest in making or using a topical ophthalmic formulation containing moxifloxacin for the treatment and prevention of bacterial infections. Based on the then-available information, such a person of ordinary skill would have expected such a formulation to be inferior to current therapies such as Ciloxan®, especially as to the treatment of sight-threatening *Pseudomonas aeruginosa* infections that were a major focus of ophthalmic anti-infective therapy.

12.   Moreover, a person of ordinary skill would have been concerned about the potential toxicity of a topical ophthalmic formulation containing moxifloxacin, both because of the toxicity of quinolone molecules as a class and the lack of sufficient published toxicity data relating to moxifloxacin, and would have been dissuaded from developing such a formulation on this basis as well. See, e.g., Ex. 4 (Rubinstein, History of Quinolones and Their Side Effects, Chemother 47(suppl3):3-8 (2001)).

13.   In addition, there was a concern amongst practitioners in the field that important ocular pathogens were increasingly resistant to the existing fluoroquinolone therapies, and that this resistance would extend to new quinolone therapies. Ex. 5 (Hodge, Frequency of Recovery of Ciprofloxacin-Resistant Ocular Isolates Following Topical Ciprofloxacin Therapy, Invest. Ophthalmology & Vis. Science, 36(4): 754-662 (1995)); Ex. 6 (Maffett, Ciprofloxacin-resistant

Bacterial Keratitis, Am. Journal Ophthalmol. 115(4):545-46 (letter to Ed.) (1993)); Ex. 7

(Chaudhry, Scleral Buckle Infection with Ciprofloxacin-Resistant *Pseudomonas aeruginosa*,

Arch Ophthalmol 116:1251 (1998)); Ex. 8 (Snyder, Ciprofloxacin-resistant Bacterial Keratitis,

Am. Journal Ophthalmol. 114:336-38 (1992)); Ex. 9 (Knauf, Susceptibility of Corneal and

Conjunctival Pathogens to Ciprofloxacin, Cornea 15(1):66-71 (1996)); Ex. 10 (Alexandrakis,

Shifting Trends in Bacterial Keratitis in South Florida and Emerging Resistance to

Fluoroquinolones, Ophthalmology, 107(8): 1497-1502 (2000)); Ex. 11 (Goldstein, Emerging

Fluoroquinolone Resistance in Bacterial Keratitis, A 5 year review, Ophthalmology,

106(7):1313-18 (1999)); Ex. 12 (Goldstein, Emerging Fluoroquinolone Resistance in Bacterial

Keratitis: A 5 year review, IOVS 39(4): 4951-B702 (1998)); Ex. 13 (Chaudhry, Emerging

Ciprofloxacin-Resistant *Pseudomonas aeruginosa*, Am. Journal Ophthalmology, 128(4):509-10

(1999)); Ex. 14 (Kunimoto, In Vitro Susceptibility of Bacterial Keratitis Pathogens to

Ciprofloxacin, Emerging Resistance, Ophthalmology, 106(1):80-85 (1999)); Ex. 15 (Garg,

Ciprofloxacin-resistant *Pseudomonas* Keratitis, Ophthalmology, 106(7): 1319-23 (1999)); Ex. 16

(Hwang, Fluoroquinolone Resistance in Ophthalmology and the Potential Role of Newer

Ophthalmic Fluoroquinolones, Survey Ophthalmology 49(2):S79 (2004)); Ex. 17 (Blondeau,

Fluoroquinolones: Mechanism of Action, Classification, and Development of Resistance, Survey

of Ophthalmology, 49(2):S73-78 (2004)). Though some of these papers were published after

September 30, 1998, the issue of emerging resistance of ocular pathogens to fluoroquinolones

was of great concern to practitioners in the field well before that time.

      14.   A person of ordinary skill in the art would have expected moxifloxacin to work

through the same pathway as ciprofloxacin, ofloxacin, and other fluoroquinolones. Accordingly,

the skilled artisan would have considered moxifloxacin unlikely to be suitable to treat infections

caused by certain strains that had become resistant to existing quinolone therapies and would have expected ocular strains to quickly become resistant to moxifloxacin upon its use in infected patients. As a result, skilled persons were, and a person of ordinary skill in the art would have been, less interested in fluoroquinolones as a class for new ophthalmic formulations than other classes of antibiotics, including (for example) oxazolidones. See, e.g., Ex. 18 (Zurenko, Oxazolidinone antibacterial agents: development of the clinical candidates eperezolid and linezolid, Expert Opin Investig Drugs. 6(2):151-8 (1997)); Ex. 19 (Ysasaga, Efficacy of New Streptogramin (Synecrid) and Oxazolidinone (Linezolid) antibiotics against Vancomycin Reduced and Multi-Drug Resistant *Staphylococci* Recovered from Endophthalmitis Cultures, IOVS 42(4):1352-B665 (2001)).

15.   Surprisingly, Alcon's topical ophthalmic formulation containing moxifloxacin Vigamox® has numerous clinically beneficial properties that could not have been predicted based on information that was publicly known in September, 1998.[1] By way of example, moxifloxacin ophthalmic formulations have desirable pharmacokinetic properties in the eye that demonstrate a substantial and entirely unexpected improvement over the therapies in use at the September 30, 1998 priority date. The ability of a quinolone ophthalmic formulation to treat or prevent an infection was known to depend on the concentration of the compound at the site of the infection. Moxifloxacin is able to penetrate and achieve high concentrations in the cornea

---

[1] Vigamox®, like most topical ophthalmic formulations, is an eyedrop. Though other forms of topical ophthalmic formulations (such as ointments) exist for other purposes, in the area of treating and preventing bacterial infections in the cornea and aqueous humor, the products are predominately, if not exclusively, eyedrops. Topical ophthalmic formulations other than eyedrops (such as ointments) are not as practical in the treatment of corneal infections or the prevention of infections after intraocular surgery. For that reason, I will use the terms "topical ophthalmic formulation" and "eyedrop" synonymously in this report.

and in the aqueous humor—two areas of the eye where infections are traditionally most sight-threatening and difficult to treat.

16. Among the infections that can be prevented using topical ophthalmic moxifloxacin as a result of its surprising pharmacokinetic properties are infections inside the eye, including infections caused by *Pseudomonas aeruginosa* that a person of ordinary skill in the art in 1998 would have considered among the most important for a new therapy to prevent, and for which topical ophthalmic moxifloxacin would have appeared unsuitable based on the available literature. In short, the desirable and unpredictable pharmacokinetic properties of topical formulations of moxifloxacin in the eye compensate for the compound's reduced activity in vitro against *Pseudomonas aeruginosa* as compared to ciprofloxacin and contribute to clinical utility. Ex. 20 (Aliprandis, Comparative efficacy of topical moxifloxacin versus ciprofloxacin and vancomycin in the treatment of *P. aeruginosa* and ciprofloxacin-resistant MRSA Keratitis in Rabbits, Cornea 24(2):201-205 (2005)); Ex. 21 (Schlech, Overview of the Potency of Moxifloxacin Ophthalmic Solution 0.5% (VIGAMOX®), Survey Ophthalmology 50(Supp.1)S7-15 (2005)); Ex. 22 (Thibodeaux, Quantitative comparison of fluoroquinolone therapies of experimental Gram-negative bacterial keratitis, Current Eye Research 28(5):337-42 (2004)). .

17. In addition, the surprising penetration of topical ophthalmic formulations of moxifloxacin contributes to an enhanced ability to treat corneal infections (keratitis), including those caused by *Pseudomonas aeruginosa* and ciprofloxacin-resistant strains of methycillin-resistant *Staphylococcus aureus* (MRSA), both of which are important pathogens. Ex. 20 (Aliprandis); Ex. 16 (Hwang); Ex. 17 (Blondeau); Ex. 22 (Thibodeaux). Also, because moxifloxacin upon topical ophthalmic administration surprisingly kills bacteria in a different way than expected—by binding both in the expected manner but also to an additional bacterial

enzyme to which ciprofloxacin and ofloxacin do not bind—resistance to moxifloxacin has not developed as expected. Ex. 16; Ex. 17; Ex. 24 (Mather, Fourth Generation Fluoroquinolones: New Weapons in the Arsenal of Ophthalmic Antibiotics, Am. Journal of Ophthalmology, 133(4):462-66 (2002)); Ex. 25 (Drlica, A Strategy for Fighting Antibiotic Resistance, ASM News, 67(1) 27-33 (2001)); Ex. 26 (Silver, Clinical Safety of Moxifloxacin Ophthalmic Solution .5% (Vigamox®) in Pediatric and Nonpediatric Patients with Bacterial Conjunctivitis, Survey of Ophthalmology, 50(1):S55-63 (2005)); Ex. 27 (Ong-Tone, Aqueous Humor Penetration of Gatifloxacin and Moxifloxacin Eyedrops Given by Different Methods Before Cataract Surgery, J. Cataract Refract Surg 33:59-62 (2007)).

      18.   Moreover, topical ophthalmic administration of moxifloxacin surprisingly is able to prevent mycobacterial infections, which are commonly associated with LASIK surgery, more effectively than ciprofloxacin. Ex. 28 (Donnenfeld, ASCRS White Paper: Management of infectious keratitis following laser in situ keratomileusis, J. Cataract Refract Surg. 31(10):2008-11 (2005)).

      19.   The topical ophthalmic administration of moxifloxacin also recently has been found to be useful in treating fungal infections, which have been implicated in infections caused by contact lenses. Ex. 29 (Munir, Clinical Response of Contact Lens-Associated Fungal Keratitis to Topical Fluoroquinolone Therapy, Cornea 26(5):621-624 (2007)). A person of ordinary skill in the art could not have predicted in 1998 that a topical ophthalmic formulation of moxifloxacin could treat such fungal infections. Ex. 30 (Alfonso, Impact of 4th Generation Fluoroquinolones on Growth Rate and Detection Time of Fungal Pathogens, Invest. Ophthalmol. Vis. Sci. 2005;46: E-Abstract 2766).

20.   In addition, given the history of toxicity associated with quinolone antibiotics, a person of ordinary skill would have been concerned in September 1998 that moxifloxacin would have unacceptable toxicity.

21.   Alcon's commercial topical moxifloxacin ophthalmic formulation, Vigamox®, has fulfilled a long-felt need in the field for a product that could help prevent intraocular bacterial infections and treat corneal infections, including those caused by gram-positive pathogens such as *Staphylococcus aureus*, gram-negative pathogens such as *Pseudomonas aeruginosa*, and quinolone-resistant pathogens. Because of its properties, the ophthalmologic community quickly adopted Vigamox®, which has become the standard of care for prevention of potentially dangerous intraocular infections, as well as for the prevention and treatment of treatment of corneal and other ocular infections. Vigamox® has provided a significant clinical benefit to numerous patients in whom it has been used for the treatment and prevention of ophthalmic infections and has received significant praise from practitioners in the field.

22.   The literature cited by Dr. Allen does not disclose a topical ophthalmic formulation containing moxifloxacin. Nor does the literature cited by Dr. Allen suggest that moxifloxacin topical ophthalmic formulations would have the desirable properties discussed above, without many of which a person of ordinary skill in the art would not have wanted to pursue such formulations. Likewise, the available literature I have reviewed does not provide any information from which a person of ordinary skill in the art could conclude that topical ophthalmic formulations of moxifloxacin would have such properties. On the contrary, the literature as a whole discloses that moxifloxacin has significantly lower activity against *Pseudomonas aeruginosa* than ciprofloxacin and other fluoroquinolones known in September 1998, which provided a significant reason not to pursue such formulations. Moreover, the

9

literature provides no reason to believe that resistance to moxifloxacin would not arise, as it did for the quinolones that previously had been developed into topical ophthalmic formulations.

23.   In addition to offering these opinions, I may respond to and comment upon the trial or deposition testimony of other experts, including Dr. Allen. I may also form new opinions that are relevant to this case, or my opinions may change based on additional information that becomes available to me after submission of this report, including new information that comes to my attention as a result of testimony or evidence that is presented at trial.

Person of Ordinary Skill in the Art

24.   I was asked to describe the qualifications of a "person of ordinary skill in the art" as of September 1998 with respect to the '830 patent. Such a person would have an MD, OD (Optometric Doctor) and/or PhD degree and training and several years of experience in the area of treating and preventing bacterial infections, including infections in the eye, and/or research concerning these subjects. In addition, such a person would be familiar with the available options for the treatment and prevention of ophthalmic infections and would be familiar with the quinolone class of antibiotics, their history, their properties, and the microbiological techniques and parameters used to assess those properties (such as activity, pharmacokinetics in the eye, and toxicity). Such a person of ordinary skill in the art would appreciate the shortcomings of the existing therapies in September 1998 for the treatment and prevention of ophthalmic bacterial infections, as well as the nature of the infections—including the pathogenic species and location of the infections—that any new topical ophthalmic formulation would have to treat. In addition, such a person of ordinary skill would have experience working or consulting with companies interested in developing products to treat ophthalmic infections and would be familiar with the clinical and scientific considerations relevant to the decision of whether or not to pursue

development of a topical ophthalmic antibiotic. Unless otherwise indicated, references to a person of ordinary skill refer to such a person as of September 30, 1998.

25.   I note that the person of ordinary skill in the art defined as by Dr. Allen would not possess much of this knowledge, experience, or expertise. In my experience, a person with the qualifications described by Dr. Allen would not be relied on, or even involved in, the decision as to whether a topical ophthalmic formulation of an antibiotic should be pursued. Nevertheless, the opinions expressed in my report would not change if Dr. Allen's definition of a person of ordinary skill in the art were adopted.

OBVIOUSNESS AND ANTICIPATION

A. The Anatomy of the Eye and Ocular Penetration

26.   Set forth below is a summary of additional background information pertaining to the opinions I will express in this case.

27.   The eye is designed to keep foreign bodies and compounds (including pharmaceutical compounds) from entering through the ocular surface. This includes microorganisms that can cause dangerous infections and interfere with vision. This important function is achieved, in large measure, by multiple heterogeneous, protective layers on the surface of the eye. Beginning on the periphery and moving inward, the surface of the eye is coated by a tear film that has three layers: the oil, aqueous, and mucus layers. The first such layer is oily, and compounds (or any other foreign specimens) that are hydrophilic (water-loving) will have difficulty surviving in and penetrating through this layer. Beneath this oily layer of the tear film is a watery layer—an environment that is difficult for hydrophobic (water-hating) compounds to survive in and penetrate. Beneath this layer is a third layer of the tear film, which contains mucus and other materials that can interfere with the penetration of foreign compounds.

11

28.   Beneath this three-layer tear film is a epithelial cell layer on the surface of the eye, which is a hydrophobic layer that hydrophilic compounds will have difficulty penetrating. Beneath this epithelial layer is a hydrophilic stromal cell layer (which hydrophobic compounds will have difficulty penetrating), beneath which is a hydrophobic endothelial cell layer (which hydrophilic compounds will have difficulty penetrating). In any of these layers, compounds unpredictably may be highly bound (and thus not available to exert effects on pathogens or continue to penetrate into deeper structures). In addition to these multiple protective layers and mechanisms, the movement of the eyelids (blinking) promotes the rapid efflux of foreign bodies or compounds from the surface of the eye to the oropharynx (nose and mouth), where they are absorbed into the blood circulation. In addition, the eye has efflux mechanisms that actively pump compounds that do penetrate into the eye back out of the eye through the membrane. This protective system—a portion of which I have summarized here—is unique among the organs of the human body; I am aware of no other organ that contains an analogous mechanism for preventing penetration and accumulation of foreign compounds.[2]

29.   In short, in order to both penetrate the ocular surface and then remain present and accumulate in ocular tissue, the topical formulation applied to the ocular surface must strike the ideal balance of size, hydrophobicity/hydrophilicity, avoidance of efflux mechanisms, and numerous other parameters (such as binding to a various substances and tissues) that are not well

---

[2] Compounds that penetrate through the cornea and conjunctiva enter the aqueous humor. The aqueous humor is turned over, i.e. removed and replaced, frequently (approximately every five hours), thus resulting in the removal of certain compounds that enter the aqueous humor into the circulation. However, compounds such as moxifloxacin that achieve high concentrations in the aqueous humor upon topical administration can accumulate in other intraocular structures (like the iris) and thereby continue to remain inside the eye despite the frequent turnover of the aqueous humor. Compounds that achieve high concentrations in the aqueous humor can continue to penetrate into the deeper structures of the eye, including the lens, the vitreous humor,

understood. Absent any data relating to ocular penetration of a compound in an ophthalmic

formulation, a person of ordinary skill in 1998 could not predict whether a particular ophthalmic

formulation containing a particular compound will strike this balance; most compounds do not

penetrate the ocular surface well—a result that is not surprising, given the numerous protective,

heterogeneous layers that must be navigated in order to do so.[3]

B. Ophthalmic Bacterial Infections in September 1998

30.   Despite this complex and efficient system that protects against the penetration and

accumulation of foreign compounds in the cornea and interior ocular tissues, bacterial infections

often are found, and must be treated, in these tissues. These infections can arise as a result of an

injury, abrasion (for example, from contact lenses), or surgical incision in the ocular surface,

which permit bacteria to infect the cornea and intraocular tissues. Indeed, after cataract surgery

for example, it is virtually inevitable that certain pathogens present on the surface of the eye, as

well as pathogens introduced during the surgery (such as pathogens present on the surgical

instrumentation) will be present in the aqueous humor and cornea. If not properly treated or

prevented, pathogens in the aqueous humor or cornea can cause serious infection in one of those

areas or migrate into areas even deeper into the eye and cause endophthalmitis.

31.   Irrespective of whether these infections remain in the cornea or aqueous humor or

migrate and cause endophthalmitis, such infections were considered at the priority date (and still

---

the retina, and the choroid. Compounds transported out of the aqueous humor (by the turnover
of the aqueous humor) enter the bloodstream via blood vessels.

[3] For this reason, one alternative to topical treatment of interior ocular infections is intravitreal
injection, in which the formulation is introduced into the interior vitreous humor by way of a
needle. Needless to say, this is not an attractive alternative, but one that may be employed if a
topical formulation cannot penetrate and accumulate at the site of infection at a sufficient
concentration to kill the bacteria.

are considered) the most important infections for a topical ophthalmic antibiotic formulation to treat and prevent. That is because infections on the surface of the eye (conjunctivitis) had been treated by existing therapies for decades, while infections in the cornea and interior ocular tissues (for example, in the aqueous humor) were not sufficiently treated and prevented by existing therapies. Exs. 5-17, 31-37. Infections in interior ocular tissues, if not adequately treated or prevented, can cause permanent impairment of vision, complete loss of vision, and even loss of an eye. See Ex. 28; Ex. 31 (Steinert, Current Therapy for Bacterial Keratitis and Bacterial Conjunctivitis, Am. Journal of Ophthalmology 112:10S-14S (1991)); Ex. 32 (Forster, The Management of Infectious Keratitis As We Approach the 21st Century, CLAO Journal, 24(3): 175-80 (1998)). Therefore, having the ability to treat surface infections would not have been sufficient for the person of ordinary skill to have been interested in pursuing an ophthalmic formulation containing a particular compound.[4]

32.   Though various species of pathogens may infect the cornea and interior tissues of the eye, among the most important and site-threatening is *Pseudomonas aeruginosa*.[5] Ex. 39

---

[4] Compounds that had been used for decades before 1998, such as sulfacetamide, were successfully treating surface infections. See, e.g., Ex. 33 (Lohr, Comparison of three topical antimicrobials for acute bacterial conjunctivitis, Pediatr. Infect. Dis. J., 7:626-29 (1988)); Ex. 34 (Olson, Challenges in Ocular Infectious Diseases and the Evolution of Anti-infective Therapy, Survey Ophthalmology, 49(1) S53-S61 (2004)) ("the more common surface infections, such as conjunctivitis, fortunately do not often lead to serious consequences and may even resolve on their own in the absence of specific anti-microbial therapy."). Thus, it was the treatment and prevention of bacterial infections beneath the surface of the eye that was the goal of a person of ordinary skill. See, e.g., Ex. 35 (Javitt, National Outcomes of Cataract Extraction, Endophthalmitis Following Inpatient Surgery, Arch Ophthalmol. 109:1085-89 (1991)); Ex. 36 (Endophthalmitis Vitrectomy Study Group: Results of the endophthalmitis vitrectomy study, Arch Ophthalmol. 113:1479-96 (2004)); Ex. 37 (Montan, Endophthalmitis after cataract surgery: risk factors relating to technique and events of the operation and patient history: a retrospective case-control study, Ophthalmology, 105:2171-7 (1998)); Ex. 31 (Steinert).

[5] *Pseudomonas aeruginosa* also is an important pathogen in other tissues, including the lungs. By contrast, certain other pathogenic species that are relevant in the treatment and prevention of ocular infections are not as important in the treatment or prevention of infections elsewhere in

(Alfonso, Ulcerative keratitis associated with contact lens wear. Am J Ophthalmol 101(4):429-33 (1986)); Ex. 10 (Alexandrakis); Ex. 11 (Goldstein); Ex. 40 (Eifrig, Endophthalmitis Caused by *Pseudomonas aeruginosa*, Ophthalmology 110(9) 1714-17 (2005)); Ex. 15 (Garg). Indeed, I note that Dr. Allen recognizes that *Pseudomonas aeruginosa* as an important ocular pathogen. Allen report at ¶ 29.

C. The State of the Art in the Treatment of Ophthalmic Infections in September 1998

33. Generally, when a topical ophthalmic antibiotic formulation is administered, the pathogenic species to be treated or prevented is unknown. Ex. 41 (Bower, Fluoroquinolones in the Treatment of Bacterial Keratitis, Am. Journal Ophthalmol. 121(6):712-15 (1996)); Ex. 42 (Masket, Preventing, diagnosing and treating endophthalmitis, J. Cataract Refract Surg. 24:725-6 (1998)); Ex. 32 (Forster). A person of ordinary skill considering whether to pursue development of a topical ophthalmic formulation in September 1998 therefore would have been concerned with the formulation's ability to treat and prevent infections in the cornea and intraocular tissues caused by all important ocular pathogens, including those caused by *Pseudomonas aeruginosa*. See, e.g., Ex. 11 (Goldstein); Ex. 10 (Alexandrakis).

34. A topical ophthalmic formulation's ability to treat and/or prevent infection generally depends on two factors: (1) the activity of the active ingredient against the infective bacterial strain (often measured by its minimum inhibitory concentration) and (2) the amount of

---

the body, and activity against such pathogens often is not reported in the non-ophthalmic literature. See, e.g., Ex. 38 (Lin, Comparative Efficacy of Topical Ciprofloxacin for Treating Mycobacterium fortuitum and Mycobacterium chelonae Keratitis in an Animal Model, Am J. Ophthalmology 117:657-62 (1994)).

active ingredient present at the site of infection, discussed above.[6] In addition, the formulation

must not cause unacceptable toxicity in the course of treating or preventing the infection. The

acceptability of a level of toxicity depends on the benefit provided by the treatment and the risk

to benefit ratio of other available options for treatment and prevention.

35.   The state of the art products for the treatment and prevention of bacterial

infections in September 1998 were Ocuflox® and Ciloxan®, topical ophthalmic formulations

containing (respectively) ofloxacin and ciprofloxacin as the active ingredient at concentrations of

.3%.[7] Ciprofloxacin is not very soluble in the aqueous solvent in which it is formulated, and

requires a more acidic (pH of approximately 4.5) environment to stay in solution. Ex. 43

(McGee, Safety of Moxifloxacin as Shown in Animal and In Vitro Studies, Survey

Ophthalmology, 50(1):S46 (2005)). The acidity of Ciloxan is uncomfortable for patients and

causes burning in the eye. In addition, when this acidic formulation containing .3%

ciprofloxacin is introduced to the neutral (pH approximately 7) environment of the ocular

surface, in which ciprofloxacin is less soluble, it can crystallize (come out of solution in solid

form), which is both uncomfortable and disconcerting for patients (who can see flecks of a white

solid on the surface of their eyes). Finally, while ciprofloxacin was quite active against most

important ocular pathogens, including *Pseudomonas aeruginosa*, it had shortcomings in

---

[6] The amount of drug at the site of infection may be measured by various pharmacokinetic parameters, including maximum concentration (Cmax), area of the compound under the curve (AUC), or simply the concentration of compound present at various timepoints.

[7] The other topical ophthalmic fluoroquinolone formulation available in the United States in 1998 was Chibroxin (.3% norfloxacin). This formulation was much less active than ophthalmic ciprofloxacin against important ocular pathogens. By 1998, the use of topical ophthalmic quinolone formulations such as Ciloxan® and Ocuflox® was the standard of care in the treatment of conjunctivitis and keratitis and the prevention of post-operative keratitis and endophthalmitis. Ex. 32 (Forster); Ex. 8 (Snyder). Other non-quinolone antibiotics also were used.

preventing certain bacterial infections in the interior tissues of the eye, such as endophthalmitis (an infection in or near the retina that causes inflammation of the inner coating of the eye and can result in sight loss) that can arise following common cataract surgery, and preventing and treating keratitis infections, including those associated with surgery. Exs. 5-17.

36.   Ocuflox® did not have the crystallization problems associated with Ciloxan®. However, ofloxacin (the active ingredient in Ocuflox®) lacked ciprofloxacin's desired activity against gram-negative bacteria (though it had slightly better gram-positive activity). In addition, Ocuflox® did not penetrate sufficiently to reach the desired concentrations to treat or prevent certain bacterial infections, including those caused by *Pseudomonas aeruginosa* (based on ofloxacin's MIC values against the infecting species). Ex. 44 (Diamond, Topical .3% ciprofloxacin, norfloxacin, and ofloxacin in treatment of bacterial keratitis: a new method for comparative evaluation of ocular drug penetration, British Journal of Ophthalmology 79:606-09 (1995)).

37.   In addition, in the late 1990s, the emerging resistance of certain ocular pathogens to treatment by Ocuflox and Ciloxan was a grave concern for persons of ordinary skill in the art. Exs. 5-17. Given this concern, especially as it relates to *Pseudomonas aeruginosa*, as well as the fact (discussed above) that bacterial ocular infections generally are treated and prevented without knowledge of the identity of the pathogen, a person of ordinary skill in the art in 1998 would only have been interested in new therapies with enhanced ability to treat and prevent all important ocular pathogens in interior tissues, which were increasingly resistant to current therapy. Indeed, the expectation of a person of ordinary skill in the art in the late 1990s was that any new fluoroquinolone ophthalmic formulation would not solve this problem of fluoroquinolone resistant bacteria, and that the solution to the problem was more likely to be

found by looking to other (non-fluoroquinolone) classes of antibiotics. See, e.g., Ex. 18 (Zurenko).

38.   A person of ordinary skill would have been interested only in a fluoroquinolone formulation that would have been expected to solve these shortcomings in the existing products and not be toxic. With regard to toxicity, the history of fluoroquinolones was discouraging, as most fluoroquinolones (both before and after 1998) proved, after human testing and use, to be unacceptably toxic. Ex. 4 (Rubinstein); Ex. 45 (Kimura, Drug-Induced Pneumonitis with Eosinophilic Infiltration Due to Tosufloxacin Tosilate, Nihon Kokyuki Gakkai Zasshi, 36:618-622 (1998) (Abstract)); Ex. 46 (June 9, 1999 FDA Advisory). Given the purposes for which the topical ophthalmic formulations at issue would be used, and the availability of other options (such as Ciloxan®), a person of ordinary skill in the art would not have been interested in developing a topical ophthalmic formulation of a compound he believed might be toxic.

39.   As noted above, I advised companies at the time (and continue to do so today) regarding what compounds may be suitable for use in an ophthalmic formulation. The issues discussed above relating to treatment of all important ocular pathogens, treatment of resistant strains, the avoidance of resistance development, ocular pharmacokinetics, and toxicity were (and still are) crucial to the decision-making process. Any decision that ignores any of these factors—let alone most of them—does not reflect the reality of how a person of ordinary skill in the art would have approached the question of whether to pursue a topical ophthalmic formulation in 1998. Put another way, a company or person of ordinary skill in 1998 would not have decided whether to pursue a topical ophthalmic formulation of a compound based on its in vitro activity against a limited set of non-ocular pathogens and a perceived ability to formulate the compound into an eyedrop.

D. The Invention of the '830 Patent

40.   The invention of the '830 patent was directed to solving the shortcomings in the currently-available therapies for the treatment and prevention of ocular infections. The '830 patent identifies some of the shortcomings addressed above, including the need to treat and prevent key ophthalmic pathogens and the development of resistance by these pathogens to the currently available quinolone therapies. Ex. 3 ('830 patent) at 1:39-53. The patent then provides in vitro data regarding the activity of moxifloxacin against certain of these pathogens, including at least one strain of *Pseudomonas aeruginosa* and one quinolone resistant strain of staphylococcus aureus. The data presented in the patent—like the data in the literature discussed below—were not encouraging, as the MIC values for the tested strains of *Pseudomonas aeruginosa* and quinolone resistant *Staphylococcus aureus* were 8.0 and 4.0, respectively, which indicate low activity.

41.   Nevertheless, despite this discouraging data and the absence of public information relating to the penetration, resistance development profile, and human toxicity of moxifloxacin and topical ophthalmic formulations of moxifloxacin, the inventors conceived of the idea of a pharmaceutically useful topical ophthalmic composition comprising moxifloxacin, or a salt thereof, in a concentration from .1 to 1.0%. It is that claimed invention that I understand Dr. Allen to opine would have been obvious and anticipated in September 1998 based on the literature on which he relied in his report.

E. The State of the Prior Art in September 1998

42.   I have reviewed the literature cited by Dr. Allen, as well as additional literature relating to moxifloxacin published by that time. The publicly available literature as of the September, 1998 priority date did not disclose any topical ophthalmic formulation containing

moxifloxacin. In particular, I disagree with Dr. Allen's opinion that U.S. Patent No. 5,607,942 (Ex. 47) described Alcon's invention. Nor would the available literature have suggested to a person of ordinary skill in the art that a topical ophthalmic formulation containing moxifloxacin would be desirable, let alone that it would possess the surprising properties that have enabled topical ophthalmic moxifloxacin to solve the widely recognized problems in the field.

43.   On the contrary, the literature and knowledge publicly available at the priority date provided minimal, if any, information regarding crucial issues that would have been relevant to the person of ordinary skill in the art's evaluation of whether to pursue a topical ophthalmic formulation containing moxifloxacin. Moreover, the information that had been published regarding moxifloxacin, taken together, would have discouraged a person of ordinary skill in the art from trying or pursuing a topical ophthalmic formulation containing moxifloxacin.

44.   In support of his conclusion that the claimed topical formulation of moxifloxacin "would have been obvious to a person of ordinary skill in the art in September of 1998," Dr. Allen relies on (1) United States Patent No. 5,607,942 patent assigned to Bayer ("the '942 patent", Ex. 47), (2) in vitro data from a 1996 poster and abstract (collectively, "the 1996 Poster", Ex. 48), and (3) two declarations from Dr. Klaus Bremm that he contends were available before September 1998 ("the Bremm Declarations", Exs. 49, 50). See Allen Report at ¶¶ 28-35 and cited exhibits. I will address each of these references in turn.

45.   As an initial matter, however, it is important to note that these references on which Dr. Allen relies constitute one small sliver of the information available to a person of ordinary skill in the art in September, 1998. The quinolone art as a whole was, at that time, focused primarily on what appeared to be more promising new compounds, such as trovafloxacin and grepafloxacin. Ex. 51 (FDA approval of trovafloxacin tablets); Ex. 52 (FDA approval of

grepafloxacin tablets); Ex. 53 (Chodosh, Efficacy and safety of a 10-day course of 400 or 600 milligrams of grepafloxacin once daily for treatment of acute bacterial exacerbations of chronic bronchitis: comparison with a 10-day course of 500 milligrams of ciprofloxacin twice a day, Antimicrob. Agents Chemother. 42(1):114-20 (1998)); Ex. 54 (Leophonte, Trovafloxacin versus amoxicillin/clavulanic acid in the treatment of acute exacerbations of chronic obstructive bronchitis, Eur. J. Clin. Microbiol. Infect Dis., 17(6):434-40 (1998)); Ex. 55 (Ball, Therapeutic Advances of New Fluoroquinolones, Expert Opinion on Investigational Drugs, 7(5):761-83 (1998). And the art relating to the treatment of ophthalmologic infections remained focused on ciprofloxacin and non-quinolone therapies that could potentially solve the problems of emerging resistance; moxifloxacin would not have been on the radar screen for persons of ordinary skill in the art in September 1998. In my view, a person of ordinary skill interested in finding a new antibiotic formulation at the priority date would have looked to Ciloxan® and the references discussing that product as a starting point; that is the closest prior art to the '830 patent.

46.   Dr. Allen's focus on the three references on which he has (without explanation) chosen to rely belies the realities of the actual interests of a person of ordinary skill, the needs and medical demands that such a skilled person was attempting to address, and the information that a person of ordinary skill in the art would have considered relevant to meeting those needs. As someone who has worked in this field for approximately three decades, in determining what topical ophthalmic formulations should be pursued, I would have been unlikely to consult a poster (such as the 1996 Poster) relating to in vitro data against non-ocular, non-clinical strains presented at a conference that had nothing to do with ophthalmology, I rarely (if ever) would have read patents like the '942 patent that focus on non-ophthalmic therapies, and I have never sought out or read declarations or other Patent Office records that were to my knowledge not

found in any searchable database. In essence, Dr. Allen ignores the literature that would have been relevant in favor of references that would have been of little interest to a person of ordinary skill in the art.

47. As for the references on which Dr. Allen relies, the '942 patent relates to a new genus of chemical compounds that I understand includes millions of species. A person of ordinary skill in the art of ophthalmology would not have been interested in the '942 patent, which does not relate to the field of ophthalmology. But if such a skill person had looked to the '942 patent, he would solely have been interested in whether it contained any information suggesting that the compounds could be useful in a topical ophthalmic formulation. In this regard, a person of ordinary skill in the art would have looked for data in the '942 patent relating to the activity of the compounds (including moxifloxacin) against ocular strains, or at least non-ocular strains of pathogens that are relevant in the eye, data relating to efficacy in treating eye infections, data relating to the toxicity of the compounds, and data relating to the penetration of the compounds into the cornea and aqueous humor.

48. A person of ordinary skill in the art would have found none of this information in the '942 patent. Indeed, the '942 patent does not even indicate that moxifloxacin is an especially important compound, as I understand that it is not within the definition of "preferred compounds" that have a chlorine or fluorine substituent at C-8, and moxifloxacin is not even one of the compounds for which synthetic examples are provided. Ex. 47 ('942 patent) at Cols. 3-4. In the absence of any such data or information, a person of ordinary skill in the art would not have understood the '942 patent's lengthy list of approximately fifty kinds of formulations on which Dr. Allen relies to apply to moxifloxacin specifically, let alone disclose that moxifloxacin

22

can be used in a topical ophthalmic formulation in the dosage range claimed in the '830 patent (which dosage range likewise is not disclosed in the '942 patent).

49.   On the contrary, the language in column 56 of the '942 patent listing potential formulations applies, on its face, to the millions of compounds of the invention in general. It does not specifically apply to any single compound. It is especially clear that the statement does not relate to moxifloxacin in particular, as no formulation of any kind is disclosed for moxifloxacin (unlike the compound of example 1, for which a tablet formulation is disclosed). A person of ordinary skill in the art certainly would not interpret the patent to mean that each of the millions of compounds of the invention can and should be used in each of the approximately 50 kinds of listed formulations (and also to treat each and every kind of infection listed in the patent)—an absolute impossibility. Dr. Allen's effort to interpret the language in column 56 of the '942 patent to disclose a particular topical ophthalmic formulation of moxifloxacin amongst a possible combination of millions of compounds and approximately 50 kinds of formulations is contrary to how a person of ordinary skill in the art would interpret this reference. Such an interpretation is especially unwarranted here, given that the '942 patent discloses not a single piece of data from which a person of ordinary skill in the art could possibly conclude that moxifloxacin would be appropriate to use in a topical ophthalmic formulation (and discloses no data or formulations for moxifloxacin whatsoever). Nor is such information disclosed in the claims of the '942 patent, which I understand are directed to moxifloxacin.

50.   The Bremm declarations and 1996 Poster on which Dr. Allen relies likewise provide no information regarding the suitability of moxifloxacin for use in a topical ophthalmic formulation. Those references do not pertain to, disclose, or otherwise suggest ophthalmologic use of moxifloxacin. The Bremm declarations and the 1996 Poster, unlike the '942 patent, do provide certain data for moxifloxacin.

23

However, the data do not relate to topical ophthalmic use of moxifloxacin, the activity of moxifloxacin against ocular strains, or its efficacy in treating ophthalmic infections.

51.   The 1996 Poster contains in vitro activity data comparing moxifloxacin hydrochloride (designated as BAY 12-8039) to its enantiomer, other salts of moxifloxacin, and other quinolone compounds. Ex. 48. The Bremm declarations contain in vitro activity data comparing moxifloxacin to two other compounds (shown in the center and right columns of the chart on page BAY FH5607942-00037). Exs. 49-50. The Bremm declarations do not contain any comparison of the in vitro activity of moxifloxacin to a standard known in the ophthalmic field, such ciprofloxacin.

52.   A person of ordinary skill in the art could reach few conclusions from this very limited set of in vitro activity data presented in the 1996 Poster and the Bremm declarations. First, the activity of the compounds was tested against only approximately 20 strains of bacteria in the 1996 Poster and a small subset of those strains in the Bremm declarations. By way of comparison, when I evaluate compounds (as I often do) for possible use in treating ophthalmic infections, my laboratory tests a compound against approximately 30 ocular strains per species (hundreds of total strains). Due to inter-strain variability and the irrelevance of many of the pathogens to ocular infections, a person of ordinary skill in the art would not draw conclusions about the activity of a compound in treating ocular infections based on just the data presented in the 1996 Poster and the Bremm declarations.

53.   Moreover, none of the data published in the Bremm Declaration, the 1996 Poster, or available literature reflects testing of moxifloxacin against ocular strains of the relevant pathogens. This testing is necessary in order for a person of ordinary skill in the art to decide to make or pursue an ophthalmic formulation of an antibiotic, because compounds' activities

24

against ocular strains of pathogens can and often do differ from their activity against non-ocular strains. See, e.g., Ex. 56 (Ohnsman, Comparison of Azithromycin and Moxifloxacin Against Bacterial Isolates Causing Conjunctivitis, Current Medical Research and Opinion, 23:2241-49 (2007)).

54.   Moreover, data relating to certain pathogens that are found in the eye, including (for example) *Mycobacterium fortuitum* and *Mycobacterium chelonae*, are absent from the data presented in the declarations and poster. Ex. 38 (Lin); Ex. 28 (Donnenfeld); Ex. 56 (Khooshabeh, A case report of Mycobacterium chelonae keratitis and a review of mycobacterial infections of the eye and orbit, Tubercle and Lung Disease, 75:377-82 (1994)); Ex. 57 (Klapper, Atypical Mycobacterial Infection of the Orbit, Ophthalmology 102(10:1536-41 (1995)); Ex. 58 (Newman, A Cluster of Cases of Mycobacterium Chelonei Keratitis Associated with Outpatient Office Procedures, Am. J. Ophthalmol. 97:344-48 (1984)).[8] In order to treat and prevent such slow-growing mycobacterial corneal and endophthalmitis infections, which persons of ordinary skill in the art considered important, a topical ophthalmic formulation would have to (1) possess high intrinsic activity against the pathogens and (2) remain in the relevant ocular tissues following administration. The references on which Dr. Allen relies do not provide data for either, and therefore would not suggest to a person of ordinary skill in the art that moxifloxacin would be useful to treat such infections in the eye.

55.   In addition, it is necessary to test compounds against recent clinical isolates, since it is those strains that the compound will have to eradicate in actual clinical practice, not the often decades-old laboratory strains that are often tested in the literature and were tested in the 1996 Poster.

25

56.   In short, the 1996 Poster and Bremm declarations present data regarding non-ocular, systemic pathogens, often from research strains, that would not have been relevant to a person of ordinary skill in the art. A person of skill in the art would not rely on in vitro data against non-ocular strains to reach any conclusion about the potential for using a compound to treat ocular infections, let alone the data presented in these references. That is the reason that artisans in this field invariably test a compound against a large panel of ocular strains (in my lab or another lab that maintains such a library of strains) <u>before</u> determining that a compound possibly could be used to treat ophthalmic infections.

57.   Likewise, most of the bacterial pathogens for which in vitro activity data are presented in the 1996 Poster and Bremm declarations are not relevant to the treatment of ocular infections, and thereby would have been especially uninteresting to a person of ordinary skill in the art.[9] By contrast, as previously noted, several pathogens that are relevant to the treatment of ocular infections are absent from the panel tested in connection with the 1996 Poster and Bremm declarations.[10]

58.   Among the pathogens for which the 1996 Poster and/or Bremm declarations presented data, the person of ordinary skill in the art would have focused on the compounds' activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. As for the *Staphylococcus*

---

[8] As far as I am aware, there were no data in any publication demonstrating the activity of moxifloxacin against these pathogens in September 1998.

[9] The Bremm declaration also provides in vivo mouse data for a systemic infection. This data would not be relevant to treatment of an ocular infection by topical ophthalmic administration.

[10] The divergence between the strains tested in connection with this poster and strains that would be relevant to a person of ordinary skill in the art is not surprising. It reflects the difference between artisans interested in treating systemic infections and artisans treating ophthalmic infections, and it provides a textbook example of why a person of ordinary skill in the ophthalmologic art would not rely on data from a such a poster in deciding whether to pursue a particular compound to treat ocular infections.

aureus activity, moxifloxacin showed enhanced activity compared to ciprofloxacin against the three tested strains, as did virtually every other newer quinolone compound tested in the same experiment, including trovafloxacin, tosufloxacin, AM1155, clinafloxacin, and sparfloxacin. Ex. 48. Therefore, this in vitro data against an extremely limited set of non-ocular staphylococcus aureus strains would have provided no reason to pursue moxifloxacin instead of numerous other quinolones for which data was presented in the 1996 Poster.[11]

59. Meanwhile, the *Pseudomonas aeruginosa* activity data in the 1996 Poster would have dissuaded a person of ordinary skill in the art from pursuing a topical ophthalmic · formulation containing moxifloxacin. That is because moxifloxacin's activity against the two tested strains of *Pseudomonas aeruginosa* was approximately eight times lower than ciprofloxacin, which was the presently available treatment.

60. In addition, other fluoroquinolones were available at the time that did not lose as much activity against *Pseudomonas aeruginosa* as ciprofloxacin. Examples of such quinolones included trovafloxacin, clinafloxacin, gemifloxacin, grepafloxacin, and temafloxacin. Ex. 59 (Woodcock, In Vitro Activity of Bay 12-8039, A New Fluoroquinolone, Antimicrobial Agents and Chemotherapy, 41(1):101-06 at 102 (1998)); Ex. 60 (Fass, In Vitro Activity of Bay 12-8039, a New 8-Methoxyquinolone, Antimicrobial Agents and Chemotherapy, 41:1818-24 at 1821 (1997); Ex. 55 (Ball); Ex. 48 (1996 Poster). Indeed, the 1996 Poster on which Dr. Allen principally relies demonstrates that trovafloxacin, tosufloxacin, AM-1155, clinafloxacin, and sparfloxacin had significantly better activity against the tested strains of *Pseudomonas*

---

[11] As discussed above, a person of ordinary skill in the art would not have limited a search for a compound to treat ophthalmic infections to quinolones. On the contrary, given the development of resistance to ciprofloxacin, and the assumption that similar resistance to other quinolones would emerge rapidly upon their introduction, persons of ordinary skill in the art would have focused preferentially on other classes of antibiotics. Ex. 19 (Ysasaga); Ex. 18 (Zurenko).

aeruginosa than moxifloxacin, while improving on ciprofloxacin's activity against the relevant gram-positive pathogens. To be clear, a person of ordinary skill in the art would not have had reason to pursue topical ophthalmic formulations of these compounds either, in part because (as explained in more detail below) of concerns relating to pharmacokinetics and toxicity. But even if a person of ordinary skill in the art were to take these factors out of the equation (as no skilled person possibly would) many quinolones would have been more attractive options for topical ophthalmic formulations than moxifloxacin. In other words, if Dr. Allen were correct that a person of ordinary skill in the art would have been motivated to use topical ophthalmic formulations containing quinolones that demonstrated activity against important ocular pathogens, such a skilled person would have used such formulations containing, among others, enoxacin, trovafloxacin, clinafloxacin, gemifloxacin, grepafloxacin, and temafloxacin. Revealingly, to my knowledge, no one ever pursued a topical ophthalmic formulation containing these compounds, and I know of no evidence that any of these compounds ever were developed for topical ophthalmic use. That is not surprising, given the concerns and lack of knowledge regarding the pharmacokinetic properties, resistance development profiles, and toxicity of those compounds.

61. A person of ordinary skill in the art would have looked at the data presented the Bremm declaration and 1996 Poster (if at all) in conjunction with other references disclosing data for moxifloxacin, and with a view toward the goals of topical ophthalmic antibiotic therapy discussed above. The rest of the public literature available at that time confirmed the belief that moxifloxacin was significantly less active than ciprofloxacin and other quinolones against *Pseudomonas aeruginosa*. See Ex. 59 (Woodcock); Ex. 55 (Ball); Ex. 60 (Fass).

28

62.   As mentioned above, *Pseudomonas aeruginosa* is a virulent, sight threatening infection in the eye and was a major concern of skilled persons. As ocular strains of *Pseudomonas* that were resistant to ciprofloxacin began to emerge, a person of ordinary skill in the art would have been dissuaded from pursuing a topical ophthalmic formulation of a compound that was significantly less active than ciprofloxacin against this crucial pathogen. Accounting for the enhanced activity of moxifloxacin against certain gram-positive pathogens such as staphylococcus aureus, and ignoring all of the other relevant factors discussed in this report (which a person of ordinary skill would not have done), moxifloxacin would have been considered a sideways step in the fight against ocular pathogens. And because the bacteria continually mutate and become more difficult to eradicate, a sideways step is a backwards step.[12] In part for this reason, artisans working in the field expressed skepticism upon learning of Alcon's decision to pursue a topical ophthalmic formulation containing moxifloxacin. See Ex. 61 (Callegan, Antibacterial Activity of the Fourth-Generation Fluoroquinolones Gatifloxacin and Moxifloxacin Against Ocular Pathogens, Advances in Therapy 20(5):246-52 (2003)).

63.   A person of ordinary skill in the art in 1998 would not have been interested in pursuing a topical ophthalmic formulation of a compound, such as moxifloxacin, which on the basis of the available literature would have been expected to treat and prevent intraocular *Pseudomonas* infections less efficaciously than topical ophthalmic ciprofloxacin. The example of the compound linezolid illustrates this principle. Linezolid is a compound from a newer class of non-quinolone antibiotics called oxazolidinones that skilled artisans were considering for

---

[12] The data in the Bremm declarations is no more encouraging with respect to *Pseudomonas aeruginosa*. The only data relating to *Pseudomonas* are an activity comparison with the two compounds shown in the center and right columns of the chart on page BAY FH 5607942-000037, neither of which was known in the ophthalmic field. Without any frame of reference,

29

topical ophthalmic use, due to the growing incidence of quinolone-resistant ocular infections. Ex. 19 (Ysasaga); Ex. 18 (Zurenko). My laboratory tested linezolid (as well as another non-quinolone compound called synecrid) against a panel of ocular pathogens, which revealed improved activity against gram-positive pathogens compared to vancomycin (and for that matter ciprofloxacin). However, these compounds were significantly less active than ciprofloxacin against gram-negative pathogens, including *Pseudomonas aeruginosa*. Because the data suggested that the compound would have been less effective in treating ocular infections caused by *Pseudomonas aeruginosa* than the current therapies, linezolid was not pursued for ophthalmic use.[13]

64.   Likewise, neither the 1996 Poster nor the other literature available at the priority date provided any indication that moxifloxacin would be suitable to solve the problem of growing quinolone resistance. A person of ordinary skill in the art would have expected that moxifloxacin would bind to and kill bacteria in the same manner as ciprofloxacin, as both compounds belonged to the quinolone class of antibiotics. Accordingly, a person of ordinary skill in the art would have expected that the growing tide of quinolone resistant strains would, soon after exposure to moxifloxacin, become resistant to moxifloxacin as well. Indeed, as explained above, the frequency of quinolone resistant ocular strains was rapidly increasing as of September 1998 and was a significant concern for skilled persons in the art, and the expectation that moxifloxacin would not be able to solve this problem would have led the person of ordinary skill away from pursuing a topical ophthalmic formulation of moxifloxacin. Ex. 5-18.

---

this data would have been meaningless to a person of ordinary skill in the art. In any event, moxifloxacin was less active than those comparator compounds.

[13] Indeed, if linezolid had not been a member of a new class of antibiotics with a different mechanism of action, thus providing a possible solution to the problem of enhanced quinolone resistance, it likely never would have been considered for topical ophthalmic use.

65.   Similarly, neither the 1996 Poster nor the other literature available at the priority date demonstrated or created any expectation that moxifloxacin would be useful in the prevention and treatment of corneal infections associated with surgery or the prevention of endophthalmitis following surgery. As discussed above, these areas were of great concern to artisans in the field at the time, given the serious nature of those infections, the frequency of corneal and cataract surgeries, and the deficiencies of the available therapies in treating and preventing such infections. Ex. 35 (Javitt); Ex. 36 (Endophthalmitis Vitrectomy Study Group); Ex. 37 (Montan); Ex. 31 (Steinert).

66.   Indeed, the treatment and prevention of those infections was the purpose for which skilled artisans were looking for new topical therapies. As discussed above, a compound must penetrate into the cornea and aqueous humor and remain there over an extended period of time in sufficient concentrations in order to be useful for these purposes. And because such infections are treated and prevented without knowledge of the identity of the pathogen causing them, the concentration in the cornea and aqueous humor must be sufficient to eradicate infections caused by all important ocular pathogens, including *Pseudomonas aeruginosa*. Ex. 41 (Bower); Ex. 42 (Masket); Ex. 31 (Steinert); Ex. 32 (Forster).

67.   The ability of a topical ophthalmic formulation to prevent corneal infections and endophthalmitis cannot be predicted or expected on the basis of the data on which Dr. Allen relies. That is because concentrations of the compound in the cornea and aqueous humor over time following topical administration will dictate its utility in treating and preventing corneal infections and preventing endophthalmitis, and there existed no published data at the priority

date to suggest that moxifloxacin could be useful for these purposes.[14] A person of ordinary skill would not—and cannot—take ocular pharmacokinetics out of the equation in deciding whether a topical ophthalmic formulation should be pursued. And the Bremm declarations, the 1996 Poster, and the rest of the literature relating to moxifloxacin available at the priority date provided no information regarding the ability of moxifloxacin to penetrate into and remain in the cornea and aqueous humor.[15] Given the absence of any information regarding these pharmacokinetic properties of topical ophthalmic formulations of moxifloxacin, especially in view of the compound's significantly reduced *Pseudomonas aeruginosa* activity compared to ciprofloxacin, a person of ordinary skill in the art would not have expected that topical ophthalmic formulations of moxifloxacin could treat and prevent corneal and endophthalmitis infections, including those caused by *Pseudomonas aeruginosa*, as well as currently available therapies.

68.   Additionally, a person of ordinary skill in the art at the priority date would have been very concerned about the toxicity of moxifloxacin. Persons with expertise and experience in antibacterial toxicity invariably are consulted and provide input before a decision is made to

---

[14] The concentration of compound over time in these areas of the eye will in turn depend on numerous complex pharmacokinetic factors, including but not limited to corneal penetration, protein binding, iris-ciliary body binding, and retention time in the aqueous humor. To my knowledge, there was no data for topical ophthalmic moxifloxacin relating to any of these metrics as of September 1998.

[15] As discussed above, the eye is a unique organ with regard to its physiology of preventing penetration through the surface and quickly removing those compounds that do penetrate. The ability of a compound to achieve high tissue concentrations in non-ocular tissues is not predictive of its ability to do achieve and maintain high concentrations in the cornea and aqueous humor, where ocular pathogens must be treated and prevented. Based on my previous experience working with ophthalmic quinolone formulations, I would not have expected an ophthalmic formulation of moxifloxacin to penetrate into and remain present in the aqueous humor and cornea in substantially higher concentrations than other ophthalmic quinolone formulations.

pursue a topical ophthalmic formulation. Though that is not my particular area of focus,[16] I was

aware at the priority date that quinolones as a class were (and continue to be) notoriously toxic,

which was an issue of significant concern for persons of skill in the art. See, e.g., Ex. 4

(Rubinstein). In addition, I am aware that there was little data available regarding the safety of

moxifloxacin in humans.[17]

69.   The particular toxicity that will arise with a quinolone compound often will not be

known until it has been tested and used in thousands or millions of patients. Until such use

either identifies the toxicities associated with the compound or, far more rarely, demonstrates a

lack of significant toxicity, it is impossible to know whether the toxicity will manifest itself upon

topical ophthalmic administration of a formulation containing the compound. By way of

example, topical ophthalmic formulations containing compounds such as the antibiotic

chloramphenicol were associated with serious adverse events, including death, which

significantly curtailed their use. See Ex. 62 (Fraunfelder, Fatal Aplastic Anemia Following

Topical Administration of Ophthalmic Chloramphenicol, American Journal of Ophthalmology

93:356-60 (1982)); Ex. 63 (Fraunfelder, Systemic Reactions to Ophthalmic Drug Preparations,

Medical Toxicology 2:287-93 (1987)).

70.   Upon topical ophthalmic treatment, a compound that penetrates the eye is

absorbed via mucus membranes and travels directly to the heart. A toxicity in the heart therefore

---

[16] I understand that Dr. George Zhanel focuses his research in this area (among others) and is
submitting a report that discusses the state of knowledge regarding the toxicity of quinolones in
general and moxifloxacin in particular. I may defer to and rely on Dr. Zhanel on those topics.

[17] The Bremm declaration addresses the relative phototoxicity of various quinolones, including
moxifloxacin. However, phototoxicity is but one of many toxicities about which a person of
ordinary skill in the art would be concerned, given the history of quinolones causing (among
other things) seizures, psychotic events, tendinopathy, convulsions, renal failure, cardiotoxicty,
and coagulopathy. Ex. 4 (Rubinstein).

could be a serious concern for a person of ordinary skill in the art considering a topical

ophthalmic formulation, and such toxicity, in the form of a potassium channel blockage that can

lead to arrhythmia and death, had been associated with the fluoroquinolone sparfloxacin. Ex. 4

(Rubinstein). Likewise, grepafloxacin, which was a compound that had been approved and used

in numerous patients before the priority date, was determined (post-approval) to cause significant

blockage of the same cardiac potassium channel in the heart and lethal arrhythmias.[18] Ex. 4

(Rubinstein); Ex. 52 (FDA approval of grepafloxacin tablets). I am aware of no effort to pursue

or develop a topical ophthalmic formulation containing grepafloxacin.

71. In the absence of significant safety data in humans, a person of ordinary skill in

the art would have had no reason to believe, at the priority date, that moxifloxacin did not have

dangerous toxicity like sparfloxacin, temafloxacin, and other quinolones. In the face of such

uncertainty, a person of ordinary skill in the art would have weighed the risks of using a

particular compound in a topical ophthalmic formulation against the rewards of doing so. Here,

the topical formulation at issue would be used frequently (if not predominately) as a prophylaxis

in patients who are not infected—a use in which toxicity is especially important to avoid, lest an

otherwise healthy patient develop a serious condition or die as a result of preventive treatment

administered during (sometimes) elective eye surgery. Moreover, the existing therapies such as

Ciloxan®, despite the drawbacks discussed herein, were largely successful in treating and

preventing most infections. Accordingly, a person of ordinary skill in the art would not have

been interested in a topical ophthalmic formulation containing a compound expected to be toxic.

That is especially the case in the absence of any history of safe topical ophthalmic use that could

---

[18] A prolongation of the QT interval and resulting arrhythmia had been associated with topical
ophthalmic administration of the drug timolol maleate, an antiglaucoma drug. Ex. 63
(Fraunfelder).

assuage a skilled artisan's fear that the toxicity would present upon such administration. The literature is replete with examples of quinolone compounds that have been in development for systemic use by numerous companies over the last twenty years. To the best of my knowledge, at the priority date, not a single one of those compounds had been developed for use in a topical ophthalmic formulation before it demonstrated systemic safety by both clinical tests that provided the basis for FDA approval and widespread post-approval use in the population as a whole. That is no coincidence. In the absence of such clinical experience and safety data, a person of ordinary skill in the art at the priority date would have been dissuaded from pursuing or using a topical ophthalmic formulation containing moxifloxacin due to concerns about toxicity.

72.   Indeed, my experience with the compound trovafloxacin confirms this view. Trovafloxacin was an active quinolone compound that was approved for systemic use before the priority date, following what I understand to be Phase III clinical studies in approximately 7,000 patients. See, e.g., Ex. 51 (FDA approval of trovafloxacin tablets); Ex. 54 (Leophante); Ex. 46 (FDA June 9, 1999 Advisory). As discussed above, the data for trovafloxacin against non-ocular strains demonstrated its improved activity against gram-positive pathogens and a retention of activity against gram-negative pathogens (including *Pseudomonas aeruginosa*) as compared to ciprofloxacin. Though no company requested that we do so, my laboratory tested the activity of trovafloxacin against a panel of ocular *Pseudomonas aeruginosa* strains. Ex. 64 (Song, *Pseudomonas aeruginosa* in vitro corneal isolate sensitivity to oflox, cipro, and trova: A comparative study, Am. J. Ophthalmology 131:795-96 (2001)). That testing revealed that, against corneal isolates of *Pseudomonas aeruginosa*, trovafloxacin had activity comparable to ciprofloxacin.

73.   Despite the demonstrated activity of trovafloxacin against systemic and ocular pathogens, no company to my knowledge pursued a topical ophthalmic formulation containing trovafloxacin. If a company had interest in such a formulation, I expect that they would have approached me for the data underlying the paper I published. That never occurred. It is likely that the systemic toxicity of trovafloxacin and the absence of demonstrated safety upon topical ophthalmic administration dissuaded artisans in the field from pursuing a topical ophthalmic formulation containing trovafloxacin. It is certain that promising activity against systemic pathogens alone—even if far more promising than moxifloxacin's activity—is not nearly enough for a person of ordinary skill in the art to have reason to pursue a topical ophthalmic quinolone formulation. If it were, then persons of ordinary skill in the art would have made and used topical ophthalmic formulations containing, among others, enoxacin, trovafloxacin, clinafloxacin, gemifloxacin, grepafloxacin, and temafloxacin. I have no knowledge that any artisan pursued a topical ophthalmic formulation containing these compounds.

74.   Taken together, the literature available at the priority date would not have provided a person of ordinary skill in the art with a reason to make or use a topical ophthalmic formulation containing moxifloxacin. The literature did not provide any description of a topical ophthalmic formulation containing moxifloxacin, and the publicly available information regarding moxifloxacin provided no indication that a topical ophthalmic formulation containing the compound would be suitable to meet the goals in the art at the time. In particular, there existed no data regarding the efficacy or activity of moxifloxacin against ocular strains, and the data relating to systemic strains both did not include certain bacteria important in the eye and strongly suggested that moxifloxacin would be unsuitable to treat and prevent *Pseudomonas* infections that were of great concern to persons of ordinary skill in the art. There likewise

existed no data relating to the ocular pharmacokinetics of moxifloxacin, without which a person of ordinary skill in the art would not have expected that moxifloxacin would provide improved treatment and prevention of the infections that were of most interest—corneal infections and endophthalmitis. The literature also presented virtually no information regarding the toxicity of moxifloxacin in humans and provided no reason to believe that moxifloxacin would avoid the development of resistance to quinolones that was emerging in the late 1990s. In short, the literature at the priority date lacked information regarding most of the issues a person of ordinary skill in the art would have considered relevant to the question of whether a topical ophthalmic formulation of moxifloxacin should be pursued, and the information the literature did provide was quite discouraging. Looking at the art as a whole, a person of ordinary skill would not have had reason to make, use, or otherwise pursue a topical ophthalmic formulation of moxifloxacin at the priority date. The invention of the '830 patent runs contrary to the prevailing views in the art and was a major breakthrough in the field of preventing and treating ocular bacterial infections.

F. The Properties of Topical Ophthalmic Moxifloxacin

75.   As a practitioner who regularly researches, prescribes and administers topical antibiotic formulations, I am familiar with the properties of the available formulations, including Alcon's Vigamox® product containing moxifloxacin. I understand that for purposes of discussing the properties of Vigamox® and other ophthalmic formulations, I may consider information and literature that was not publicly available at the priority date.

76.   Topical ophthalmic moxifloxacin has properties that a person of ordinary skill in the art could not have expected at the priority date, which contribute to it meeting the needs in the field discussed above and explain why it has become a very successful commercial product.

37

77.   As explained above, because a compound must reach the site of infection in sufficient concentrations and at the right time in order to treat or prevent infection, the ocular pharmacokinetics of a topical ophthalmic formulation is crucial in the treatment and prevention of the corneal infections and endophthalmitis about which a person of ordinary skill in the art would have been concerned in September 1998. Put another way, quinolones are concentration-dependent antibiotics, so their ability to treat and prevent infections will depend on the concentrations achieved at the relevant time of infection at the relevant site of infection. Surprisingly, topical ophthalmic moxifloxacin is able to penetrate into and remain in the cornea and aqueous humor in concentrations that far exceed those achieved by topical ophthalmic formulations containing other quinolones. See, e.g., Ex. 65 (Kim, Ocular penetration of moxifloxacin .5% and gatifloxacin .3% ophthalmic solutions into the aqueous humor following topical administration prior to routine cataract surgery, Current Medical Research and Opinion 2H(1):93-94 (2005)); Ex. 66 (Kim, Aqueous Penetration and Biological Activity of Moxifloxacin .5% Ophthalmic Solution and Gatifloxacin .3% Solution in Cataract Surgery Patients, Ophthalmology 112:1992-96 (2005)); Ex. 67 (Solomon, Aqueous Penetration of Gatifloxacin, Moxifloxacin, and Ciprofloxacin, Ophthalmology 112:466-69 (2005)); Ex. 68 (Robertson, Invest. Ophthal. Vis. Sci. 2004;45:ARVO E-Abstract 4906); Ex. 27 (Ong-Tone); Ex. 22 (Thibodeaux). For the reasons discussed above, the favorable pharmacokinetics of topical ophthalmic moxifloxacin would not have been expected in September 1998.

78.   The surprising pharmacokinetic properties of topical ophthalmic moxifloxacin are important in its clinical use. As a result of its unexpected pharmacokinetic properties, topical ophthalmic moxifloxacin is useful to treat and prevent corneal infections and prevent endophthalmitis, including such infections caused by *Pseudomonas aeruginosa*. Ex. 20

(Aliprandis); Ex. 27 (Ong-Tone); Ex. 70 (Leaming, Practice styles and preferences of ASCRS members—2003 survey, J. Cataract Refract Surg 30:892-900 (2004)); Ex. 21 (Schlech); Ex. 22 (Thibodeaux); Ex. 16 (Hwang); Ex. 17 (Blondeau); Ex. 71 (Kowalski, The Prevention of Bacterial Endophthalmitis by Topical Moxifloxacin in a Rabbit Prophylaxis Model, Research in Vision and Ophthalmology, 1467/B363 (2003)). Indeed, absent this significant enhancement in penetrating and remaining in, among other tissues, the aqueous humor and cornea, compared to other topical ophthalmic formulations containing fluoroquinolones, topical ophthalmic moxifloxacin would be far less clinically useful and would not have become a product frequently used by artisans in the field to treat corneal infections and prevent corneal infections and endophthalmitis associated with surgery. In other words, an important reason that topical ophthalmic moxifloxacin is useful to treat and prevent the infections about which a person of ordinary skill in the art was interested in 1998 is its enhanced pharmacokinetic properties, about which a person of ordinary skill in the art would have had no knowledge at the time.

79.   Remarkably, topical ophthalmic moxifloxacin is able to achieve far higher concentrations in internal ocular tissues than other quinolones despite the fact that it uniquely lacks a preservative such as benzylkonium chloride (BAC). Preservatives are known to degrade the corneal epithelial layer and thereby promote penetration of active compounds. Ex. 72 (Tripathi, Cytotoxicity of Ophthalmic Preservatives on Human Corneal Epithelium, Lens and Eye Toxicity Research 9(3&4):361-75 (1992)); Ex. 73 (Kim, Evaluation of the effects of topical ophthalmic fluoroquinolones (FQ) on the cornea using in vivo confocal microscopy. Poster presented at the Annual Meeting of the Association for Research in Vision and Ophthalmology; May 5, 2003. Poster B263). In an apples-to-apples comparison in which formulations with moxifloxacin and its comparator quinolones either both contained or both lacked a preservative,

the significant enhancement in tissue concentrations achieved by moxifloxacin following topical administration (discussed above) would be even more pronounced.

80.   In addition, and quite surprisingly, topical ophthalmic moxifloxacin has been useful in treating mycobacterial infections that have become a significant concern for persons of ordinary skill in the art. Ex. 74 (Lee, Fourth-Generation fluoroquinolones in the treatment of mycobacterial keratitis after laser-assisted in situ keratomileusis surgery, Can. J. Ophthalmol. 40(6):753-56 (2005)); Ex. 75 (Alfonso, Ophthalmic Infections and Their Anti-infective Challenges, Survey of Ophthalmology 50(1):S1-S6 (2005)); Ex. 76 (Abshire, Topical antibacterial therapy for mycobacterial keratitis potential for surgical prophylaxis and treatment, Clin. Ther. 26:191-96 (2004)).

81.   Indeed, as laser-assisted in situ keratomileusis (LASIK) surgery became more common in the late 1990s and early 2000s, the incidence of corneal infection caused by atypical mycobacteria increased substantially. Post-LASIK corneal infection, which can be a devastating surgical complication, became such a concern that the American Society of Cataract and Refractive Surgery (ASCRS) commissioned a survey to determine the causes and incidence of such infections and issued a Special Report to discuss the results of the survey and the published reports in the literature. The report concluded that atypical mycobacteria were the most frequent cause of post-LASIK corneal infection, and that organisms are "not responsive" to the then-available therapies (which included Ciloxan®, but did not include topical ophthalmic moxifloxacin). Ex. 77 (Solomon, Special Report, Infectious Keratitis after laser in situ keratomileusis: Results of an ASCRS Survey, J. Cataract Refract Surg. 29(10):2001-06 (2003)). A few years later, the ASCRS performed another survey, after Vigamox® was introduced, which

"revealed a significant decrease in atypical mycobacteria, with only 2 cases reported."[19] Ex. 28 (Donnenfeld). One important reason for the decreased incidence of post-LASIK corneal infections was the availability and use of Vigamox®. Id.

82.   In addition, and quite unexpectedly, topical ophthalmic moxifloxacin was able to meet the need in the field for a therapy that could treat quinolone-resistant ocular strains and not succumb to growing resistance upon its introduction. As explained above, quinolone-resistant ocular pathogens were a major concern for artisans in the field, and the expectation would have been that such resistance would rapidly emerge in response to the introduction of a new quinolone therapy, thereby severely limiting the utility of the therapy. Exs. 5-17. The solution to this problem of resistance was to find a therapy with a new mechanism of action in killing bacteria. Ex. 18 (Zurenko). Moxifloxacin, as another quinolone in the same class as ofloxacin and ciprofloxacin, would not have been expected to have a different mechanism of action in killing bacteria. Surprisingly, it does, by binding to both the bacterial DNA gyrase and topoisomerase IV enzymes, which accounts for moxifloxacin's continued success in treating resistant strains of ocular pathogens. Ex. 17 (Blondeau) (explaining moxifloxacin's dual binding mechanism of action that inhibits both DNA gyrase and topoisomerase IV, which can limit the emergence of fluoroquinolone resistance); Ex. 78 (Pestova, Intracellular targets of moxifloxacin: a comparison with other fluoroquinolones, J. Antimicrob Chemother 45:583-90 (2000)); Ex. 16 (Hwang 2004); Ex. 27 (Ong-Tone). As a result, while a mutation in one bacterial gene may cause resistance to quinolones such as ciprofloxacin, two mutations (which will occur with far less frequency) often are required for a strain to be resistant to moxifloxacin.

---

[19] Prophylaxis [treatment] with moxifloxacin was not used in either of those cases.

83. Moreover, Vigamox® surprisingly has proven useful in treating fungal infections, which recently have been implicated in infections caused by contact lenses. Ex. 29 (Munir); Ex. 30 (Alfonso). I am not aware of any data published at the priority date demonstrating or providing any expectation that topical ophthalmic moxifloxacin would have this property.

84. In addition, as discussed above, a person of ordinary skill in the art would have focused on the issue of safety in evaluating whether to make and use a topical ophthalmic formulation of moxifloxacin. The safety of moxifloxacin in both systemic and topical ophthalmic formulation, after millions of uses, is quite surprising. Indeed, moxifloxacin, almost uniquely among fluoroquinolones of interest before, at, and after the priority date, has proven to be quite safe. Ex. 4 (Rubinstein). For the reasons discussed above, this safety is important to the clinical use and success of topical ophthalmic moxifloxacin. In fact, topical ophthalmic moxifloxacin is so safe that it has even been demonstrated to be sufficiently safe for use in children. Ex. 26 (Silver).

85. As a result of these properties, Alcon's topical ophthalmic moxifloxacin formulation has met a long-felt need in the industry for a safe product that can treat and prevent corneal infections and prevent endophthalmitis more effectively than existing therapies. These properties have also caused artisans in the field to adopt topical ophthalmic moxifloxacin rapidly and praise it widely. The properties of topical ophthalmic moxifloxacin I have discussed in this report also explain why Vigamox® has become a market leader and a commercially successful product.

September 19, 2007

Eduardo C. Alfonso, M.D.

43